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CYTOLOGICAL AND EVOLUTIONARY IMPLICATIONS OF ABERRANT CHROMOSOME BEHAVIOR IN THE HARLEQUIN LOBE OF SOME PENTATOMIDAE (HETEROPTERA)

By

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With 63 Figures in the Text

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I. Introduction

The regular occurrence of aberrant chromosomal behavior in certain groups of animals has frequently been the subject of study. One need mention only the formation of oligopyrene and apyrene sperms in some *Mollusca* and *Lepidoptera* to recall the length to which such aberrations may go. Quite different, although equally bizarre, are the meiotic maneuvers of the chromosomes as seen in some species in the heteropteran Family *Pentatomidae*. This unusual behavior is restricted to a single one of the compartments or lobes that constitute the testis. It is to be emphasized that, in the species concerned, every testis contains such a "harlequin lobe" and that it is always the same lobe, structurally speaking, that is affected. There can be no doubt that the factors which bring about the development of a harlequin lobe are genetically determined, but such a conclusion does not go very far in telling us how the effects are produced. As a first step in the analysis of these conditions,

a brief survey of the species involved as well as the morphology of the testis would seem to be called for, after which the cytological aspects will be considered.

I take pleasure in expressing my gratitude to Dr. HERBERT RUCKES of the American Museum of Natural History, who has frequently laid aside other pressing work to identify specimen collected by me and has, as occasion warranted, described and named those that were new.

II. Methods and Material

In the great majority of the species the gonads were fixed in Sanfelice, but in a few specimen collected in earlier years, Allen's Bouin was employed. Heidenhain's haematoxylin and Feulgen were both used in the study of every species, the former being indispensable in bringing out the spindle structure whereas the latter is extremely useful in following the chromosomes during the meiosis and especially in the meiotic prophase. In all the illustrations the sex chromosomes have been drawn in solid black wherever they could be recognized. Drawings were made from sections stained with haematoxylin unless otherwise noted.

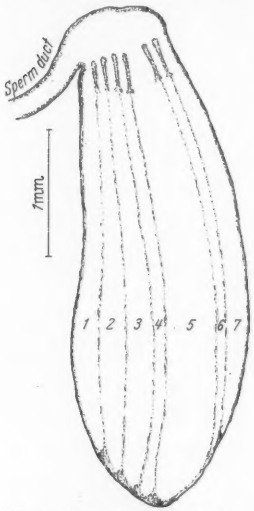


Fig. 1. The structure of the testis in *Loxa picticornis*. Lobe No. 5 is the harlequin lobe; No. 4 and 6 are "large cell" lobes. The rest are normal

The 21 species here under discussion all belong to the heteropteran Family *Pentatomidae* and the Subfamily *Pentatominae*; 3 Tribes are involved: the *Halyini*, the *Discocephalini*, and the *Pentatomini*. The material was collected in the course of 20 odd years and, as the table indicates, all the species are tropical in habitat except *Loxa florida*, which is subtropical.

The general anatomy of the female and male gonads in the *Heteroptera* is well known (BONHAG 1955, 1958; BOWEN 1922b). Each gonad consists of a number of compartments or lobes and, in the testis, these lobes are held together throughout their length by a surrounding, stout sheath. Within this sheath the individual lobes are separated from their neighbors by very thin, cellular partitions which appear to be extensions of the outer connective tissue. In most *Pentatomidae*, the testicular lobes are arranged side by side, more or less in one plane, but in a few species they are bunched more irregularly (MONTGOMERY 1898, 1910; BOWEN 1922b). BOWEN has arbitrarily numbered these lobes consecutively, considering the one nearest the side at which the sperm duct enters as No. 1. This practical scheme has been followed throughout the present paper (Fig. 1).

Table. *List of 21 Species with a Harlequin Lobe*

Family: *Pentatomidae:* Subfamily: *Pentatomidae:* Tribe: *Halyini*	Collecting Locality
Alitocoris parvus (DISTANT)	Barro Colorado Island, Panama
Alitocoris schraderi SAILER	Cairo and Turrialba, Costa Rica
Macropygium reticulare FABRICIUS	Turrialba, Costa Rica
Melanodermus tartareus STAL	Los Diamantes, Costa Rica
Moncus obscurus DALLAS	Barro Colorado Island, Panama
Schraderia cinctus RUCKES	Los Diamantes and Turrialba, Costa Rica
Schraderia hughesae RUCKES	Turrialba, CostaRica
Tribe: *Discocephalini*	
Ablaptus amazonus STAL	Turrialba, Costa Rica
Agaclitus dromedarius STAL	Turrialba, Costa Rica
Architas pudens DISTANT	Barro Colorado Island, Panama
Dinocoris rufitarsus RUCKES	Barro Colorado Island, Panama
Mecistorhinus panamensis RUCKES[1]	Barro Colorado Island, Panama
Mecistorhinus sepulcralis FABRICIUS	Piracicaba, Brazil
Mecistorhinus tripterus FABRICIUS	Turrialba, Costa Rica
Neodine macraspis PERTY	Cairo, Costa Rica
Tribe: *Pentatomini*	
Brachystethus rubromaculatus DALLAS	Turrialba, Costa Rica
Loxa flavicollis DRURY	Los Diamantes, Costa Rica; Barro Colorado Island, Panama
Loxa florida VAN DUZEE	Southern Florida, USA
Loxa picticornis HORVATH	Turrialba, Costa Rica; Barro Colorado Island, Panama
Mayrinia variegata (DISTANT)	Siquirres, Costa Rica
Pseudovoplitus longirostris RUCKES	Barro Colorado Island, Panama

[1] This species was earlier identified for me as *Mecistorhinus melanoleucus* and was so considered in a former paper (SCHRADER 1946b). It now appears that it represents a new species, *Mecisthorhinus panamensis* RUCKES. The Tribe *Discocephalini* to which it belongs, is a highly interesting group of pentatomids. It has on occasion been given the rank of a Subfamily and is again so considered in the most recent papers of RUCKES (1959a, 1959b). But until the publication of Dr. RUCKES' extensive study of the discocephaline *Pentatomidae* which he is now undertaking, I have thought it best to retain the tribal ranking of the *Discocephalini* which has been assigned to them by most authors since KIRKALDY's 1909 catalogue.

The number of lobes may differ in the various species and there is some difference of opinion concerning the number of lobes that may be considered basic in the *Heteroptera*. For the present we may agree with the eminent entomologist WEBER (1930, 1933) that the normal number is 7. Deviations from this number nearly always take a downward direction and only in a single species, the discocephaline *Dinocoris rufitarsus*, is there an increase — to 8 lobes.

In a great many pentatomids there is a constant difference in the size of the cells found in the different lobes. BOWEN observed that in most such cases of "polymegaly", one of the lobes is characterized by very small spermatocytes. There also may be lobes that carry unusually large spermatocytes and in nearly all such cases there are two of these. When both types of lobes are present, the two "large cell" lobes always flank the "small cell" lobe, one on each side. If in such instance the normal number of 7 lobes comprises the testis, the "small cell" lobe is always No. 5, and the "large cell" lobes are No. 4 and 6. It is the lobe corresponding to No. 5 which becomes the harlequin lobe. The rest of the lobes carry spermatocytes of normal dimensions and if there are size differences among them, they are very slight.

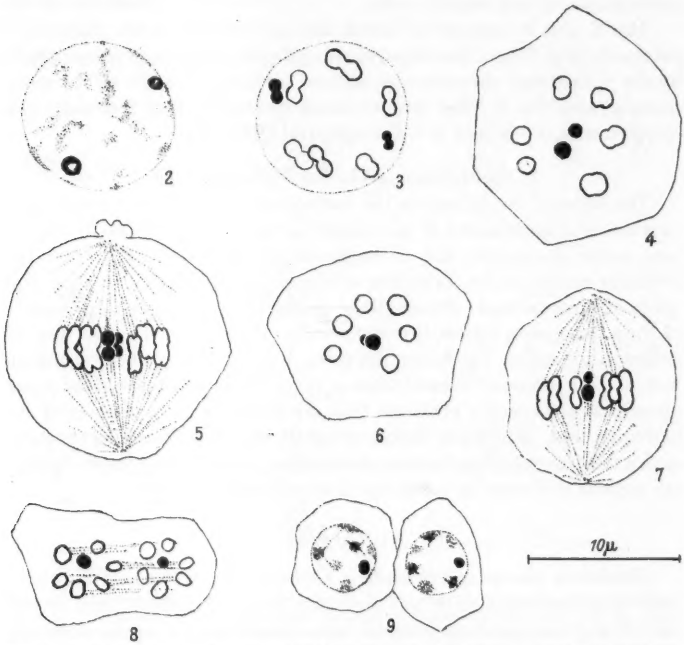
III. Cytology

1. Normal Spermatogenesis

The normal spermatogenesis in the *Heteroptera* has frequently been studied; it was first described in detail by WILSON in his classic paper of 1912 and various aspects of it have been discussed by other workers since that time. A renewed, detailed treatment is therefore hardly called for.

In all the species here under discussion except one, the diploid chromosome number is $14 = 6$ pairs of autosomes $+ X + Y$. The exception is *Macropygium reticulare* which has a compound X of 2 components and therefore has a diploid complement of 15 in the ♂. The drawings of the normal meiosis, given here for ready reference, were made from cells in the normal lobes of *Schraderia cinctus*. The course of the meiosis in these normal lobes is quite orthodox. A marked heteropycnosis characterizes the sex chromosomes in all the prophase stages of the male. Even in the confused stage when the autosomes become more or less diffuse and can no longer be distinguished as individual chromosomes, the X and Y remain in a heteropycnotic condition and are recognizable at once (Figs. 2 and 3). In the majority of the nuclei, the two sex chromosomes are joined in a single, spherical body at this time, but in every cyst there is always a number of perfectly normal cells in which such a union has not taken place. It is this latter type

of cell which is figured here as well as in later drawings of this stage, largely because the number of heteropycnotic chromosomes present in the prophase has some relevance to the analysis of the harlequin lobe. In any case, the X and Y always separate again, long before the first meiotic metaphase is reached.



Figs. 2—9. *Schraderia cinctus* — Normal Lobe. Fig. 2. Confused stage, showing the heteropycnotic X and Y (Feulgen). Fig. 3. Late diakinesis, X and Y drawn solid black. Fig. 4. First metaphase, polar view. 6 Autosomal bivalents in circle around the sex chromosomes. X ist the larger of the sex chromosomes. Fig. 5. First metaphase, side view. Fig. 6. Second metaphase, polar view. Fig. 7. Second metaphase, side view. X and Y undergoing "touch and go" pairing. Fig. 8. Second anaphase, with X and Y segregating from each other. Fig. 9. The two types of spermatids, carrying 6 autosomes and either X or Y

As in the first metaphase of other *Pentatomidae*, the autosomal bivalents tend to be arranged in a circle around the centrally placed sex chromosomes (Figs. 4 and 5), though departures from this arrangement are not rare. In the ensuing division, the X and Y divide equationally.

In the second metaphase the circular arrangement of the autosomal dyads around the sex chromosomes repeats the general configuration

of the first metaphase, but the placement of the chromosomes is more precise and exceptions to the arrangement are decidedly rare (Fig. 6). It is to be noted that these metaphase configurations, with the autosomes disposed in a ring at some distance from the polar axis, are seen only during meiosis; there is no indication of such a placement in spermatogonial and somatic cells.

The X and Y undergo a "touch and go" pairing in the vertical or polar axis (Fig. 7) and then separate to opposite poles, each accompanied by the 6 daughter chromosomes derived from the division of the autosomal dyads (Fig. 8). The two resulting spermatids therefore carry the complements 6 + X and 6 + Y respectively (Fig. 9).

2. Spermatogenesis in the Harlequin Lobe

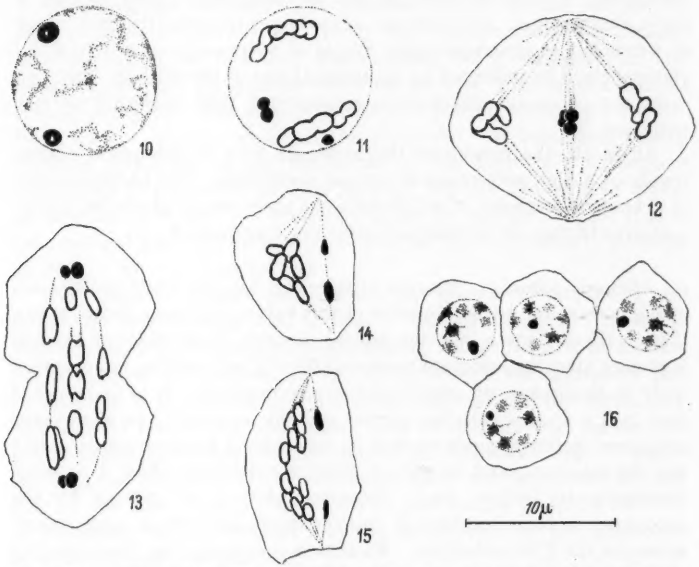
The unusual conditions in the harlequin lobe find their cytological expression in aberrations of the meiotic process. In 19 of the 21 species here under discussion, the consequence of such deviations from the orthodox meiosis is the formation of spermatids that carry an abnormal quota of chromosomes. Although the aberrations vary in certain features of their expression in the different species, they follow a rather similar course in the main. The following, more detailed survey of the situation will give a more exact idea of what is involved and will also point more directly at the specific problems that are posed by the existence of the harlequin lobe. The three Tribes involved will be discussed separately, such a treatment having certain advantages in considering the evolutionary aspects that may be taken up on some future occasion.

a) Halyini

Schraderia cinctus and *Schraderia hughesae*. These two halyinids have harlequin lobes that show less deviation from the normal than any of the other pentatomids considered here. In *Schraderia cinctus* the early and mid prophase stages parallel those of the normal lobes in every respect, as does the confused stage with its vesiculated but highly heteropycnotic sex chromosomes (Fig. 10). In late diakinesis the 6 condensed autosomal bivalents appear in the "dumb bell" form that is so characteristic of the bivalents or tetrads as they appear in all pentatomids. But just before the breakdown of the nuclear membrane there is a tendency for them to come together in twos and threes (Fig. 11) — rarely in clusters greater than that. This deviation from the normal meiosis persists into the first metaphase where, in addition, these autosomal aggregates are further removed from the centrally placed sex chromosomes than is the case in the normal configuration (Fig. 12).

The tendency of the autosomal bivalents to adhere to each other may or may not be responsible for the irregularity of the ensuing anaphas-

movement. While the sex chromosomes divide smoothly, there is a marked asynchrony in the division of the autosomal bivalents of which some dyads may still linger close to the equator when others have already almost reached the poles (Fig. 13). Nevertheless the normal number of dyads is finally distributed to the two second spermatocytes in nearly all cases.



Figs. 10—16. *Schraderia cinctus* — Harlequin Lobe. Fig. 10. Confused stage (Feulgen). Fig. 11. Late diakinesis, showing some clumping of autosomal bivalents. Fig. 12. First metaphase; distance of bivalents from polar axis larger than in normal lobe. Fig. 13. First anaphase; X and Y dividing smoothly but autosomal dyads moving asynchronously. Fig. 14. Early anaphase of second division. Fig. 15. Mid anaphase of second division; 6 autosomes going to each pole. Fig. 16. Spermatids; all carrying 6 autosomes and either X or Y

The configuration of the second spermatocyte division departs more widely from the normal than does the first. While the X and Y go through the usual "touch and go" pairing in the polar axis, the 6 autosomal dyads aggregate in a rather loose cluster that is subject to a slight, lateral displacement (Fig. 14). But again, this division like the first results in a regular distribution of chromosomes. The X and Y segregate quite normally to opposite poles while the autosomes, despite their failure to form the characteristic ring or any sort of equatorial plate, undergo a division which results in a distribution of 6 autosomes to

each spermatid (Fig. 15). Just as in case of the normal lobes there are thus two types of spermatids: 6 + X and 6 + Y.

It is surprising that the peculiarities of these meiotic divisions do not result more frequently in errors of chromosome distribution. That such errors are not too common is shown especially well by the early spermatids. Of 50 spermatids that permitted quite reliable chromosome counts only 4 departed from the normal complement, each only by a single chromosome. As in normal lobes, larger irregularities may occur at times but in somewhat later stages of spermateleosis where a deviation would be indicated by an unusual size of the nucleus, only two cysts out of many dozens were encountered that showed such size variation.

All in all, the meiosis in the harlequin lobe of *Schraderia cinctus* results in a high percentage of normal spermatids. The harlequin lobe of *Schraderia hughesae* (of which only one male was available for study) conforms to that of *Schraderia cinctus* in every respect.

Alitocoris schraderi. MARTIN (1953) who has described the meiosis in the normal and harlequin lobes of this halyinid in some detail, states that in the harlequin lobe the diploid number of chromosome threads is present after the leptotene stage and that "if any pairing has occurred prior to that stage, no evidence of it now remains". It is to be noted that in this species both the normal and the harlequin lobe show some autosomal heteropycnosis as well as the normal heteropycnosis of the sex chromosomes, and several of MARTIN's drawings show 3 or more heteropycnotic bodies. As in *Schraderia* there is a tendency for the autosomes to come together in twos or threes and this is most clearly shown in the first metaphase. MARTIN is convinced that the clumping occurs at random and does not necessarily involve homologous univalents. Here too, the first division witnesses a distribution of equal numbers of autosomes to each pole in the great majority of cases, and such distribution indicates that each univalent divides equationally. In the course of this division the chromosomes begin to lose their compact appearance and "begin a partial return to a more diffuse state", an alteration that had earlier been noted also in the harlequin lobes of several *Discocephalini* (SCHRADER 1946b).

In the second division of *Alitocoris* the deviation from the normal becomes more pronounced. The autosomes are then aggregated in a clump in which they can no longer be distinguished as individuals, and this clump suffers a distinctly greater displacement from the polar axis than is the case in *Schraderia*. In the anaphase, when the X and Y segregate to opposite poles, the autosomal mass elongates and one of the larger autosomes becomes dissociated from it, and begins its pole-

ward journey. Somewhat later some other autosomes in varying number follow it and the resulting spermatids carry a highly variable number of chromosomes.

Alitocoris parvus differs from these proceedings in the harlequin lobe in no discernible respect.

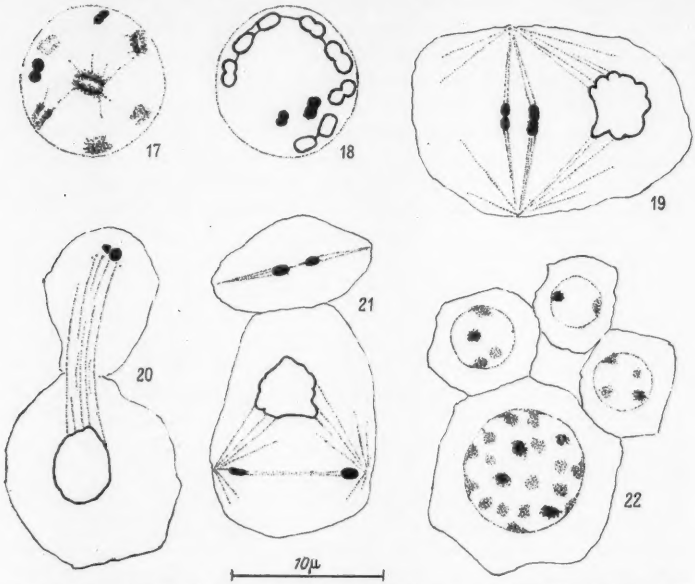
Macropygium reticulare. The spermatogenesis of this species has been reported by SRIVASTAVA (1957) who, though one of his primary interests lay in the fact that *Macropygium* has a compound X, also describes the chief steps in the harlequin meiosis. SRIVASTAVA believes that at pachytene there is some evidence of pairing, though his illustrations are not entirely convincing on this point. At best, such pairing involves only some of the autosomes or else there is a subsequent desynapsis of the bivalents thus formed. Though SRIVASTAVA makes no mention of it, my own observations on the species indicate that in the confused period there is some autosomal heteropycnosis in addition to that of the sex chromosomes.

Just prior to prometaphase, when the nuclear membrane is still intact, the chromosomes tend to form a chain in the equatorial region. But this chain is disrupted in prometaphase and at the first metaphase the autosomes are gathered in two or three separate aggregates just as they are in *Schraderia* and *Alitocoris*. SRIVASTAVA, like SCHRADER and MARTIN before him, also makes note of the fact that during this period the chromosomes becomes somewhat "diffuse looking". Despite the irregular clumping of the autosomes some are distributed to each pole in the first division. SRIVASTAVA was unable to follow the sex chromosomes in this division and the distribution of the autosomes is evidently quite irregular.

In the second division the autosomal clumping is more extreme and at the metaphase all are gathered in a single cluster. SRIVASTAVA believes that this cluster is displaced little from the polar axis. However, this displacement is as great as it is in *Schraderia* and he states that the sideward distortion in the shape of the clump indicates that forces of lateral displacement are active in *Macropygium* too. As might be expected the number of chromosomes in the spermatids is quite variable.

Moncus obscurus. Here the deviations in the harlequin lobe are decidedly more extreme than in the preceding five species. The pairing, however, appears to be normal; at any rate in the early diakinesis 6 autosomal bivalents are indubitably present (Fig. 17). Slightly later a disunion has begun in several bivalents though, usually, not more than three are thus affected. In late diakinesis the autosomes move into the equatorial region and there generally form a chain that maintains

close contact with the nuclear membrane until it disintegrates. The two sex chromosomes are almost never included in this chain nor do they touch the nuclear membrane (Fig. 18). It should be stated that such chain formation sometimes occurs also in the normal lobes but is there less frequent. Probably the movement to the equatorial



Figs. 17—22. *Moncus obscurus*. Fig. 17. Early diakinesis; 6 autosomal bivalents and heteropycnotic sex chromosomes. Fig. 18. Late diakinesis; some desynapsis of autosomal bivalents. Fig. 19. First metaphase; side view. Fig. 20. Late first anaphase; sex chromosomes at lower pole obscured by clumped autosomes. Fig. 21. Second anaphase; one cell containing only X and Y, the other also including clump of autosomes. Fig. 22. Spermatids with great range of chromosome numbers; most of the chromosomes in large spermatid have undergone disunion of chromatids

region is alike in both types of lobes and in the course of it the chromosomes must frequently come into contact with each other. The higher incidence of chains in the harlequin lobe may mean no more than that such contacts are there less easily broken again.

After the disintegration of the nuclear membrane all the autosomes are gathered in one clump and, in the establishment of the first metaphase, this clump is moved away laterally from the polar axis. The displacement is a considerable one and is often so forceful that the cell wall is bulged out before it (Fig. 19).

In the division that follows, the sex chromosomes divide equationally, but the autosomal mass remains intact and is included entire in one of the two second spermatocytes. Usually the X and Y in this second spermatocyte become lost to view in this autosomal aggregate during telophase whereas the sex chromosomes in the sister cell are of course quite conspicuous (Fig. 20).

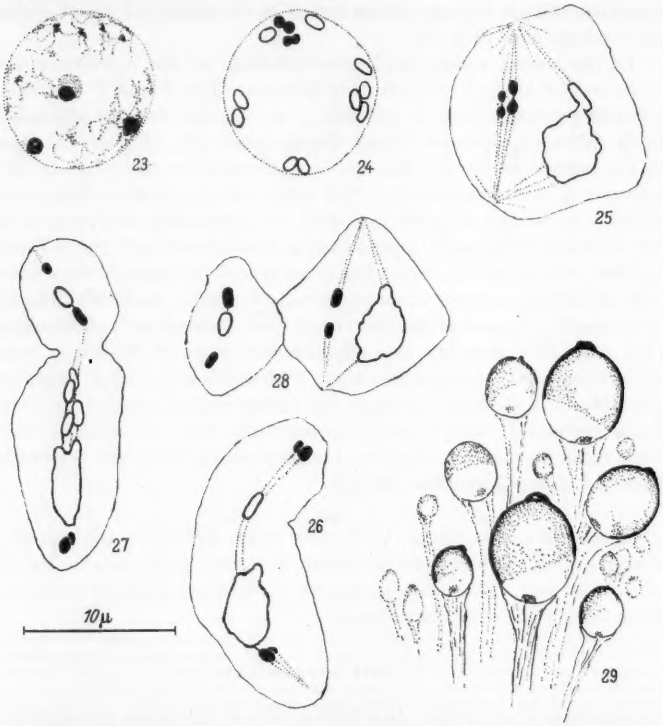
In the second spermatocyte that contains all the autosomes, the configuration of the first division is repeated. The X and Y take their customary position close to the polar axis while the clumped autosomes again suffer an extensive lateral displacement (Fig. 21). At anaphase all the autosomes usually pass into one spermatid with one of the two segregating sex chromosomes. The sister cell therefore receives only either X or Y, just as is the case with the spermatids that result from the division of the second spermatocyte that carried only the XY pair to start with (Fig. 21). Since the large spermatids usually show more than the diploid number of chromosome bodies there must be a disunion or separation of some of the chromatids that compose each chromosome (Fig. 22). This probably also explains why none of the many, very small spermatids contain less than 2 chromosomal bodies when the expectation is that only a single sex chromosome is carried by them. Some spermatids with 4 and 6 chromosomes were encountered which must mean that on rare occasions 1 or 2 autosomes disengage themselves from the clump during the division.

Melanodermus tartareus. With some minor differences the preceding description of the harlequin meiosis in *Moncus* is applicable to *Melanodermus* as well. These two species thus represent extreme departures from the normal course of meiosis.

b) Discocephalini

Agaclitus dromedarius. In addition to that of the sex chromosomes, there is marked heteropycnosis of one autosome or autosomal pair. Heteropycnosis is present also in some other autosomes but the extent of this is very slight (Fig. 23). Prophase pairing is either incomplete or else there is some desynapsis before diakinesis. The chain formation so frequently seen in other species, almost never occurs here (Fig. 24). The first metaphase shows a lateral displacement of the cluster of autosomes that is not as great as in several other species. Almost simultaneously with the initiation of the anaphase movement of the X and Y, a single autosome leaves the clump (Figs. 25 and 26) and is always included in a second spermatocyte with the equational halves of the sex chromosomes. Somewhat later several other autosomes also

move out of the clump but these laggards apparently rarely reach the pole with their predecessor and the cleavage constriction leaves them in the other second spermatocyte with the rest of the autosomes and a pair of sex chromosomes (Fig. 27).



Figs. 23—29. *Agaclitus dromedarius*. Fig. 23. Confused stage; X, Y, and one autosome or autosomal pair strongly heteropycnotic, with some heteropycnosis in other autosomes (Feulgen). Fig. 24. Late diakinesis; some clumping, but no chain formation (Feulgen). Fig. 25. Early first anaphase; one large autosome moving out of clump to upper pole with the sex chromosomes. Fig. 26. Mid anaphase of first division; large autosome moving to upper pole with X and Y. Fig. 27. Late anaphase of first division; large autosome near upper pole, but cleavage constriction indicating that rest of autosomes will remain in lower cell. Fig. 28. Second anaphases; one large autosome and sex chromosomes in one cell, other cell containing clump of autosomes and sex chromosomes. Fig. 29. Larger spermatids developing normally, but smaller ones becoming irregular and staining faintly

In the division of the second spermatocyte that contains the autosomal clump, the latter is displaced laterally at metaphase, as usual. In the other second spermatocyte where in most cases only a single autosome is included with the sex chromosomes, this autosome usually

accompanies the X in the division (Fig. 28). The four spermatids resulting from these two second spermatocytes thus all carry one of the sex chromosomes, but one of them usually has only a single autosome in addition and another contains all the rest of the autosomes.

All the spermatids take the first steps in the process of spermateleosis but it is very doubtful whether the spermatids, that include only very few chromosomes, go very far in their development. At the stage shown in Fig. 29 only the larger spermatids appear to be undergoing a normal development whereas the smaller nuclei are becoming vague in outline and can not be recognized in later stages.

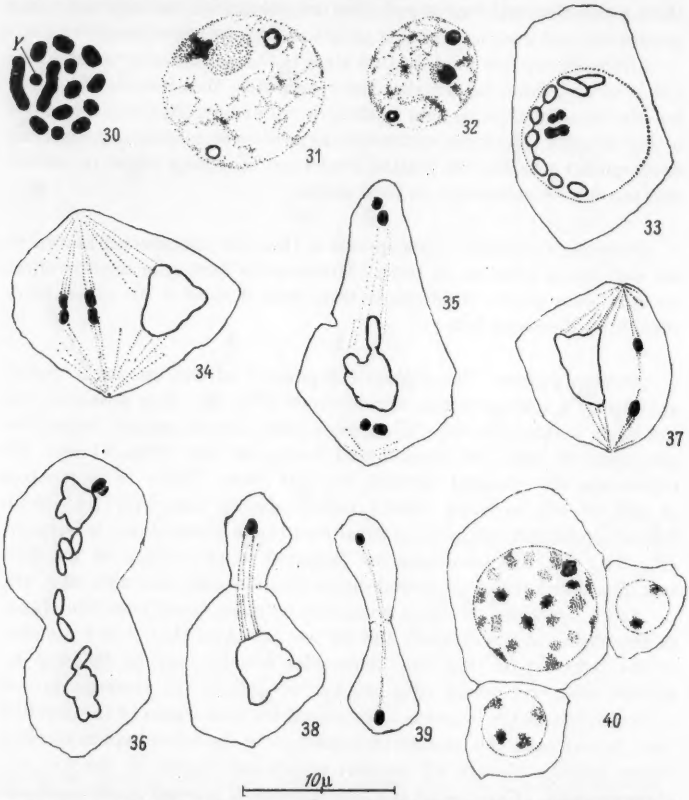
Dinocoris rufitarsus. This species is the only pentatomid known to me that has 8 lobes in its testis. However its harlequin meiosis offers no significant points of difference from that of most of the other discocephalines discussed here.

Architas pudens. The diploid complement of this species is distinguished by a very large pair of autosomes (Fig. 30). It is probable that the large, heteropycnotic, chromosome body that is present during the prophases of both the normal and harlequin lobe (Figs. 31 and 32) represents the bivalent formed by this pair. There is desynapsis of this as well as some other bivalents during diakinesis; and chain formation, shortly before the nuclear membrane breaks down, is frequent (Fig. 33). All the autosomes are included in one clump at the first metaphase and the usual lateral displacement occurs here also (Fig. 34).

As in *Agaclitus* one large autosome detaches itself from the clump in the course of the division and its size indicates that it is a member of the largest pair (Fig. 35). Somewhat later it may be followed by several other autosomes (Fig. 36) but not rarely the cleavage furrow is formed before the laggards have passed the mid region of the dividing cell. In any case, the number of autosomes in the second spermatocytes varies greatly, though all contain equational halves of the two sex chromosomes. The second division follows the usual pattern of the discocephaline harlequin meiosis (Figs. 37, 38 and 39) and spermatids with a great range in the number of chromosomes result. Here too, large spermatids carrying more than the diploid number of chromosomal bodies occur frequently and this connotes that there is a separation of the chromatids of some of the chromosomes (Fig. 40).

Mecistorhinus panamensis, *Mecistorhinus sepulcralis*, *Mecistorhinus tripterus*, and *Neodine macraspis.* The course of meiosis in the harlequin lobe of these four species has been described in an earlier publication (SCHRADER 1946b). That of *Mecistorhinus panamensis* (called *Mecisto-*

rhinus melanoleucus in that publication) was reported in some detail and is more or less representative of the other three. A notable feature



Figs. 30—40. *Archilas pudens*. Fig. 30. Spermatogonial metaphase showing exceptionally large pair of autosomes. Fig. 31. Confused stage in "large cell' (normal) lobe; large pair of autosomes heteropycnotic as well as X and Y (Feulgen). Fig. 32. Confused stage in harlequin lobe; large pair of autosomes heteropycnotic as well as X and Y (Feulgen). Fig. 33. Late diakinesis; loose chain formation of autosomes at periphery and some disunion in the bivalents, including large one. Fig. 34. First metaphase; side view (Feulgen). Fig. 35. First anaphase; one large autosome leaving lump. Fig. 36. Late first anaphase; several autosomes undergoing mitotic movement and clumping again. X and Y hidden by autosomes at lower pole. Figs. 37, 38, and 39. Second anaphases; in cells containing autosomes as well as cell with only X and Y. Fig. 40. Spermatids carrying varying number of chromosomes; disunion of chromosomes into chromatids is evident in largest spermatid

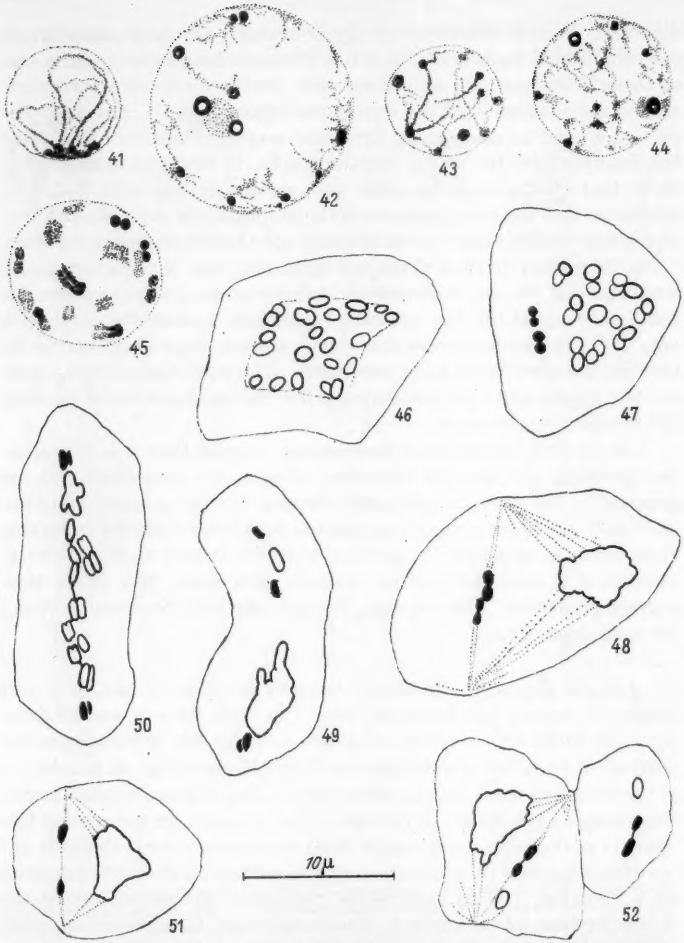
lies in the fact that here too one of the autosomal pairs is heteropycnotic during the prophase. Further, in the first division when nearly all the

autosomes become diffuse or spongy in texture, two large members of the complement remain compact like the sex chromosomes. It is one of these heteropycnotic autosomes that always leaves the autosomal cluster and it alone joins the equational halves of the X and Y at one pole. A second heteropycnotic autosome detaches itself somewhat later but finally rejoins the rest of the autosomes. It would seem more than likely that in this heteropycnotic pair we are dealing with the same autosomes that are heteropycnotic in the prophase and there is a manifest correlation between their heteropycnosis and their exceptional behavior.

In the second division of the cell containing only the heteropycnotic autosome plus the sex chromosomes, this autosome always accompanies the X into one of the two resulting spermatids, leaving the other with only a Y. The autosomes in the second spermatocyte which carries all the rest, are distributed quite irregularly. The indications are that here too the spermatids that contain very few chromosomes never produce fully formed spermatozoa.

Like several others, these four species suggest that it is not alone the clumping and possible adhesions between the autosomes that are responsible for their irregular distribution in the meiotic divisions. Obviously the heteropycnotic autosomes have little difficulty in leaving their clumped congeners, suggesting that the failure of the others to undergo a normal distribution involves alterations that affect their mitotic properties. Their spongy texture may well be visual evidence for such alterations.

Ablaptus amazonus. As usual, the early prophase of meiosis is very similar in normal and harlequin lobes. In both there is much heteropycnosis which shows clearly that not only the sex chromosomes but sections of a number of autosomes are thus affected (Figs. 42 and 44). It is this heteropycnosis that underlies the striking difference in the pachytene stage as it appears in the two types of lobes. In the normal lobe there is at that time a collocation of all the heteropycnotic elements at a restricted region of the nuclear membrane and this results in the formation of a "bouquet" — so rare in the meiosis of *Pentatomidae* (Fig. 41). A similar orientation but in a more exaggerated form occurs in certain *Edessini* (SCHRADER 1941) and is there brought about in exactly the same way. In *Ablaptus* it is a more temporary phenomenon and has already disappeared when the confused stage is reached. But highly significant is the fact that in the harlequin lobe, where the amount of heteropycnosis parallels that in the normal lobe, there is no such collocation of the heteropycnotic elements and hence no bouquet formation (Fig. 43). It is a striking demonstration of the environmental effects on chromosomal behavior.



Figs. 41—52. *Ablaptus amazonus*. Fig. 41. Pachytene stage in normal lobe, showing "bouquet" (Feulgen). Fig. 42. Confused stage in "large cell" (normal) lobe; extensive heteropycnosis of autosomes as well as sex chromosomes (Feulgen). Fig. 43. Pachytene stage in harlequin lobe, showing no "bouquet" (Feulgen). Fig. 44. Confused stage in harlequin lobe; heteropycnosis just as extensive as in normal lobe (Feulgen). Fig. 45. Early diakinesis; all autosomes present as univalents (12), and some segments that are heteropycnotic (Feulgen). Fig. 46. Late diakinesis with nuclear membrane beginning to disintegrate; most autosomes show separation of their chromatids. X and Y can not be recognized (Feulgen). Fig. 47. Prometaphase; autosomes still clustered only loosely but X and Y already in separate position (Feulgen). Fig. 48. First metaphase; side view. Fig. 49. First anaphase; one autosome going to upper pole with X and Y (Feulgen). Fig. 50. Late first anaphase; extensive movement of autosome (Feulgen). Figs. 51 and 52. Second divisions of different types

In the early diakinesis of the harlequin lobe, the heteropycnosis in some of the autosomes is still noticeable (Fig. 45). At this time also it becomes evident that the autosomes are all present as univalents and, as diakinesis progresses, the chromatids of many of these univalents become separated from each other. The result is that nuclei that are close to the breakdown of the nuclear membrane often show considerably more than the diploid number of chromosomes (Fig. 46). At this time the two sex chromosomes can not be distinguished from the autosomes which here, as in a number of other species, have become somewhat swollen and spongy in appearance.

But right after the disintegration of the nuclear membrane, the X and Y become recognizable again, for they then take a position aloof from the autosomes which gather in a cluster (Fig. 47). This cluster, loose at first, progressively becomes more compact until it constitutes a clump similar to that seen in most other harlequin lobes — a series of steps that can be followed only in Feulgen preparations. Unfortunately such preparations do not stain the spindle fibers, but the disposition of the sex chromosomes and autosomes in Fig. 47 indicates that at that time the spindle formation is already underway. These maneuvers culminate in the characteristic first metaphase configuration that is shown in Fig. 48.

As usual, the sex chromosomes divide equationally in the first division. The clump of autosomes returns to the region of the polar axis as the sex chromosomes approach the poles and in many cases its components become disengaged and are scattered in a long line as they undergo their asynchronous movement into the two daughter cells (Fig. 50). But in some other instances only a single autosome detaches itself from the autosomal mass (Fig. 49), and occasionally the latter passes entire into one of the second spermatocytes.

In the second division the autosomes are again clumped and displaced laterally (Fig. 51). The sex chromosomes, after the "touch and go" pairing, segregate smoothly to opposite poles, while the distribution of the autosomes is exceedingly variable. The end result again is a great range in the number of autosomes carried in the spermatids (Fig. 52).

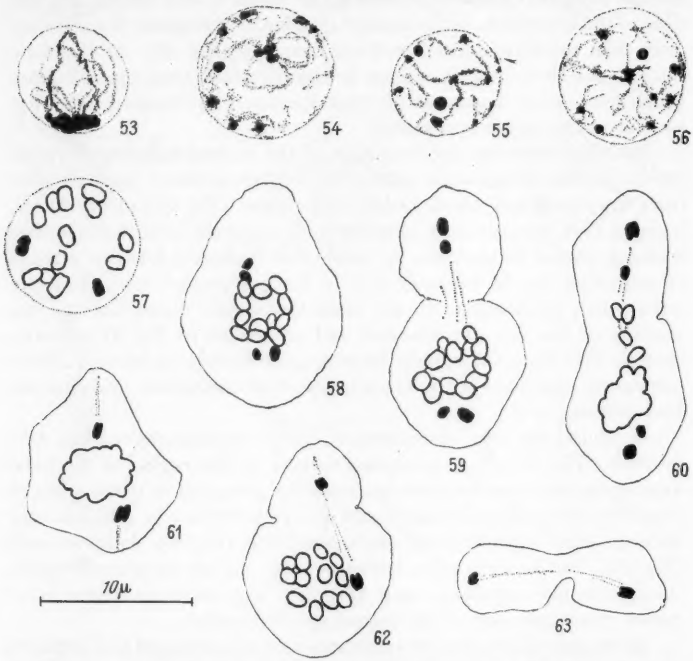
c) Pentatomini

While the *Discocephalini* display some uniformity in the type of meiotic deviation in the harlequin lobe, the *Pentatomini* are remarkable for the diversity of aberration that obtains there.

Pseudoevoplitus longirostris. Many of the aberrations in the harlequin lobe of this species are evidently similar in nature to those seen in the *Halyini* and *Discocephalini*. But it also shows some differences that

may have significance in solving some of the questions posed by the harlequin lobe.

To begin with, in both the normal and harlequin lobes of *Pseudoevoplitus* the amount of heteropycnosis considerably exceeds that of



Figs. 53—63. *Pseudoevoplitus longirostris*. Fig. 53. Pachytene stage in normal lobe, showing "bouquet". Fig. 54. Confused stage in normal lobe; exceptional amount of heteropycnosis. Fig. 55. Pachytene stage in harlequin lobe, showing absence of "bouquet". Fig. 56. Confused stage in harlequin lobe; heteropycnosis as extensive as in normal lobe. Fig. 57. Late diakinesis; 12 univalent autosomes and no chain formation. Fig. 58. Early first anaphase; autosomal cluster very loose. Fig. 59. Late first anaphase; all autosomes in lower cell. Fig. 60. Late first anaphase; some autosomes moving toward upper pole. Equational split showing in both X chromosomes. Fig. 61. Second anaphase; equational split showing in both X and Y. Fig. 62. Second anaphase; all autosomes going into lower cell with the X. Fig. 63. Second anaphase in cell containing only X and Y

any other species taken up in the present paper (Figs. 54 and 56). Apparently most, if not all, of the autosomes have large heteropycnotic sections. This is first noticeable after the zygotene stage — notoriously difficult in the *Pentatomidae* — and it is in the pachytene that the first important point of difference between the normal and harlequin lobes

presents itself. In the normal lobe there is at this time a collocation of all the heteropycnotic elements including the sex chromosomes (Fig. 53). As a result the euchromatic sections of the autosomes form a "bouquet" just as in case of *Ablaptus* described earlier, and in the *Edessini*. In *Ablaptus* the heteropycnosis is not as extensive as in the present case but obviously the same factors are involved in both species as well as in *Edessa*.

By contrast, in the harlequin lobe where an equal amount of heteropycnosis exists, there is no trace of collocation in the pachytene stage (Fig. 55). The conditions there obviously prevent such a reaction of the heteropycnotic elements or else the latter have undergone a change that is not observable under the microscope.

In diakinesis it becomes evident that the chromosomes are present as univalents, and toward the end of this period there is an irregular clumping of the autosomes. At this time too, all the chromosomes undergo some swelling and become somewhat diffuse in appearance (Fig. 57). When the nuclear membrane breaks down they gather in a very loose cluster and for a short time the sex chromosome can not be distinguished. There is no formation of a regular metaphase configuration and the beginning of the first anaphase movement is indicated by the appearance of the X and Y outside of the cluster. They apparently divide equationally within the aggregation of autosomes and the equational halves leave it to go to opposite poles (Fig. 58). There is no indication of a displacement of the autosomes and their distribution in the division is irregular. In most cases all the 12 univalents and one pair of sex chromosomes are included in one of the second spermatocytes while the sister cell receives only an X and a Y (Fig. 59). But there are some exceptions to this and occasionally a few autosomes succeed in following the sex chromosomes into the latter cell (Fig. 60).

In the second division there is again no marked displacement of the clustered autosomes. At times the X and Y undergo their segregational division somewhat to one side of the autosomes (Fig. 62) but that is not always the case and it would be difficult to decide where the polar axis is located. Certainly, the distribution of the autosomes is again irregular even though the majority of divisions shows all the autosomes passing into one of the spermatids (Fig. 62). The second spermatocytes containing only the two sex chromosomes always give rise to spermatids carrying only an X or a Y (Fig. 63).

Brachystethus rubromaculatus. A detailed description of both the normal and the harlequin meiosis was given in an earlier paper (SCHRADER 1946a). The deviations in the harlequin lobe conform in their general

course to what is seen in most other species but there are one or two exceptional features that place *Brachystethus* in a special category.

The most notable of these special features is that the autosomes undergo a perfectly normal pairing, resulting in tetradiform bivalents which are not subject to desynapsis in the later stages of the prophase. In the normal as well as the harlequin lobe the disappearance of the nuclear wall is correlated with an aggregation of all the bivalents in one cluster. But whereas these scatter again almost immediately and form the typical pentatomid metaphase configuration in the normal lobe, they remain tightly clumped in the harlequin lobe.

Through both divisions in the harlequin lobe this clump is moved as a unit and, except for the formation of some chromosomal spindle fibers, the bivalents of which it is composed give no indication of any independent mitotic activity. In no case has an autosome been seen to leave the clump in either division and if it occurs it must be rare. The sex chromosomes on the other hand, behave perfectly normally, undergoing both meiotic divisions in or close to the polar axis from which the autosomes are laterally displaced to an extreme degree at metaphase.

The persistent clumping of the autosomes and their failure to divide in either division makes for a certain degree of simplification in the final results of the meiosis. Instead of a great range in the number of chromosomes carried by the spermatids, *Brachystethus* produces only 4 types: All autosomes + X; all autosomes + Y; X; and Y. Indeed in the later stages this is probably simplified still further for it is more than likely that the tiny spermatids, which carry only a sex chromosome, never complete sperm formation. All sperms then will contain the 6 bivalents (which of course are composed of 12 univalents or 24 chromatids) and either one of the two sex chromosomes — an autosomal complement just four times as great as that carried by sperms in the normal lobes.

Obviously the case demonstrates that a perfectly orthodox pairing of the autosomes does not insure their regular and normal distribution in the harlequin meiosis.

Loxa flavicollis, *Loxa florida*, *Loxa picticornis*, and *Mayrinia variegata*. The harlequin meiosis is very similar in these four species. The conditions on *Loxa* have incited a number of investigations (BOWEN 1922a; SCHRADER 1945a, 1945b; ANSLEY 1957, 1958) but many puzzling aspects remain.

Already in early diplotene, 12 univalent autosomes are present, so that, if pairing has occurred earlier, it has been followed almost immediately by desynapsis. This has its parallel in the harlequin lobe of several other species here described, but in the confused stage there takes place

an unprecedented series of events. In every cyst of the harlequin lobe there occurs at this time an irregular fusion of spermatocyte cells as well as some amitotic divisions. This process culminates in first metaphases that contain as few as 2, and as many as 200 chromosomes. Sex chromosomes can no longer be distinguished and no lateral displacement of any kind is discernible.

Despite these irregularities in the prophase, two meiotic divisions always take place, both of which were considered equational by SCHRADER, but of which the second is regarded as reductional by ANSLEY. This second division is remarkable for the stout, Feulgen positive bridges which are formed between the chromosomes as they go to opposite poles. They clearly reflect a soft and adhesive consistency of the chromosomes in this division, not encountered in any other case. The spermatids receive a highly variable number of chromosomes and, in this respect *Loxa* parallels the other species with a harlequin lobe. However, this end result is obviously reached in an entirely different way.

IV. Discussion

The preceding account of the meiotic aberrations in the harlequin lobe in 21 species of *Pentatomidae* must be somewhat bewildering to the casual reader. Nevertheless there is a common thread that runs through the various deviations, a thread that is broken only in *Loxa* and *Mayrinia* where very special conditions are introduced at one stage of the prophase. The features that form this common thread may be outlined as follows:

a) Disruption of Meiotic Pairing

In all the species except *Schraderia* and *Brachystethus*, the pairing process of homologous autosomes or the maintenance of bivalents during prophase is in some way interfered with. In some species, such as *Alitocoris*, *Mecistorhinus* and *Loxa*, synapsis may be eliminated altogether and already at pachytene and diplotene only univalents are present. But in some other cases as for instance in *Moncus*, a meiotic pairing that appears normal in the cytological sense occurs regularly and, even as late as diakinesis, the autosomes are all in the form of bivalents. But then there occurs a desynapsis of some or all of these bivalents so that a number of univalents is present in the great majority of cases when prometaphase is reached. The cases of *Schraderia* and *Brachystethus* constitute the only exceptions, the former representing a condition intermediate between the normal and harlequin lobes; the latter a special development which not only prevents a disunion of the bivalents but also makes it impossible for them to undergo the mitotic movements that are involved in any division.

b) Clumping

In the late diakinesis of many, if not most animals, the forces that ordinarily keep condensed chromosomes separated from each other, are abrogated; and that holds also in both harlequin and normal lobes. In both types of lobes this fact is most obvious just as the nuclear membrane disintegrates, but it is first expressed prior to that when the chromosomes move to the equatorial region of the future spindle. Since the autosomes generally maintain contact with the nuclear membrane during this concerted movement, some of them are almost certain to come into contact with each other. If at that time the chromosomes are somewhat sticky, the chain formation that so frequently occurs is perhaps explicable. But such chains, which never include the sex chromosomes, are formed much more frequently in the harlequin lobe and this may simply indicate a greater adhesiveness of the autosomes there. This difference from the normal may also be reflected in the diffuse or spongy texture that the harlequin autosomes assume during this period in many of the species.

After the dissolution of the nuclear membrane in the normal lobe the chains are seen no more and indeed the metaphase once more has the chromosomes sharply separated from each other. But in the harlequin lobe this adhesiveness persists for some time; it is attested by the small aggregates of 2 or 3 autosomes in the metaphase of *Schraderia*, *Macropygium*, and *Alitocoris*, and perhaps to a greater degree by the clumping of all the autosomes in the majority of the other species.

In short, the chromosomal clumping that is of very short duration in the normal lobe, is evinced much more strongly in the harlequin lobe and there persists through both divisions.

c) Lateral Displacement

In the majority of the species the first metaphase in the harlequin lobe is remarkable for the fact that the clumped autosomes are shifted away from the mitotic axis between the two poles, in a lateral direction. Grotesque though it is in appearance, this is probably only an exaggeration of the displacement of the autosomes from the polar axis that is seen in the normal meiotic metaphases of all *Pentatomidae*. Under the less extreme conditions of the harlequin lobe in *Schraderia*, the lateral shift of the autosomes is only slightly greater than what is seen in the normal lobe where they always form a ring at a certain distance from the polar axis. In any case it betokens alterations in the harlequin autosomes that fundamentally affect their mitotic properties.

d) Distribution of Chromosomes

In no case is the anaphase movement a normal one. Even in *Schraderia* where the great majority of spermatids in the harlequin lobe receives a normal complement of chromosomes, there is much asynchrony

in their progress to the poles. In all other cases the number of chromosomes distributed to the spermatids is highly variable and sperms with an orthodox complement of chromosomes are rare.

The irregularity in the distribution of chromosomes is not solely due to the clumping and possible adhesion of the autosomes to each other. There are several cases such as *Mecistorhinus*, *Architas*, *Ablaptus*, and *Agaclitus*, where one of the autosomes experiences little difficulty in disengaging itself from the autosomal clump and undergoing a more or less normal anaphase movement. In every one of these instances, such an autosome seems to be heteropycnotic, and it thus parallels the perfectly normal mitotic activity of the sex chromosomes which are also heteropycnotic. This would seem to suggest that clumping and adhesion may indeed impede the anaphase movement to some extent but that the basic difficulty lies in the fact that the alterations in the autosomes have seriously impaired their normal mitotic activity. And further, these alterations affect heteropycnotic chromosomes much less than they do isopycnotic chromosomes.

It is not too venturesome to postulate that such changes in the chromosomes are brought about by alterations of the chemical conditions within the harlequin lobe. We already know that the various lobes of the pentatomid testis are not alike in their chemical processes, and that is true even in those species that have not developed a harlequin lobe. Thus it has been shown that in *Arvelius* the different dimensions attained by nuclei, nucleoli, and cytoplasm in the "small, normal and large cell" lobes are the result of different degrees of synthetic activity. These do not affect the DNA in the various lobes but most decidedly influence the RNA and the proteins (SCHRADER and LEUCHTENBERGER 1950, 1951). It is conceivable that when the "small cell" lobe becomes a harlequin lobe, a change in the amount of DNA may also be involved, but this possibility may be dismissed. In *Loxa*, ANSLEY (1957, 1958) found that the DNA in the nuclei of the harlequin lobe shows no quantitative difference from that of normal lobes. Similarly, the very exact "two wave length" photometric determinations of NICKLAS (paper in preparation) established that the amounts of DNA in the nuclei of the harlequin and the normal lobes in *Mecistorhinus* and *Neodine* are identical. But ANSLEY'S cytophotometric analysis of other constituents demonstrates that in *Loxa* the quantitative relations of the histones to the DNA are decidedly different in the meiotic cells of the two types of lobes. Further, ANSLEY is inclined to attribute the harlequin beharvior not only to that fact but also to changes in the amino acid composition of these histones. Obviously these investigations are only the first steps in the cytochemical analysis of the harlequin lobe but they are sufficient to show that the lobes differ in their chemistry. It is only one more

step to predicate that the changed chemical environment in the harlequin lobe has brought about chromosomal alterations that are more far reaching in the isopycnotic than in the heteropycnotic elements.

e) Heteropycnosis

The difference in the reaction of isopycnotic and heteropycnotic elements to the cellular environment in the harlequin lobe, brings up the old problem of heteropycnosis. Heteropycnosis, as the cytologist sees it, is a general term that may cover several different phenomena and, as it occurs under pathological conditions it no doubt differs fundamentally from the heteropycnosis that becomes apparent in certain chromosomes at definite stages of the life cycle. The not uncommon assumption that it represents a type of chromatin that is consistently distinct from "euchromatin" and is genetically degenerate has recently been subjected to a penetrating analysis by COOPER (1959), who showed in *Drosophila* that in its genetic properties it can not thus be relegated to the structures that are being discarded in the process of evolution. Indeed, there is ample evidence that under certain conditions some types of heteropycnotic chromatin can assume all the cytological properties of "euchromatin". One need only recall that the X chromosome, which is strikingly heteropynotic in the meiosis of male *Heteroptera*, is completely isopycnotic in the female (TROEDSON 1944). But that is not to say that the two types of chromatin are identical in composition; a chromatin that is heteropycnotic in a certain environment obviously must differ in some physico-chemical respect from one which is not. That under certain circumstances heteropycnotic elements may show a more normal behavior than isopycnotic elements is abundantly shown by the behavior of the chromosomes in the harlequin lobe.

f) Evolutionary Aspects

A consideration of the evolutionary questions raised by the existence of the harlequin lobe was published in several earlier papers (SCHRADER 1945a, 1946a, 1946b). A more detailed treatment will appear in another place and at the present time only one or two isolated points will be mentioned.

It would seem almost certain that the harlequin sperms carrying aberrant chromosome complements very rarely if ever produce viable offspring. It is therefore puzzling how so wasteful an organ could have evolved. And wasteful it is, for in the great majority of the 21 species the harlequin lobe is larger than any of the other lobes, comprising at least 2/5 of the entire testicular volume in several instances. It is possible however, that the sperms that emanate from the harlequin lobe are not entirely

useless, a utility that may be correlated with the fact that polyspermy occurs in the fertilization of many, and probably most, insect eggs. The nucleoproteins transmitted to the egg by such supernumerary sperms are no doubt absorbed and used by the developing embryo. If now, as appears probable, the harlequin sperms that carry a low number of chromosomes rarely reach full sperm development, then most supernumerary sperms that stem from the harlequin lobe will carry into the egg a greater quantity of nucleoproteins than the normal supernumeraries. Thus in the case of *Brachystethus* each such sperm would transmit four times as much nucleoprotein as a normal sperm. It is at least conceivable that herein lies a factor that may have favored the harlequin evolution.

Summary

1. In the harlequin lobe of 21 species of *Pentatomidae*, the spermatogenesis always undergoes bizarre deviations from a normal meiosis.

2. The deviations affect the meiotic pairing in most cases and also involve irregularities in the meiotic divisions which result in a highly variable number of chromosomes in the spermatids.

3. There is good evidence that the chemical conditions within the harlequin lobe differ from those in normal lobes and that they are responsible for alterations in isopycnotic chromosomes that are most strikingly manifest in their mitotic behavior.

4. Evidently the changed chemical environment has comparatively little effect on chromatin which is in the heteropycnotic state. Hence the mitotic behavior of the sex chromosomes in the harlequin lobe is normal, and autosomes that are predominantly heteropycnotic show less deviation from the normal than do isopycnotic chromosomes.

5. The radically different behavior of chromosomes in adjoining regions of the same testis is a demonstration of the fact that chromosomal behavior is, to a considerable extent, determined by their immediate environment.

6. The question of how so wasteful an organ as the harlequin lobe could have been evolved, is briefly considered.

References

Ansley, H.: A cytophotometric study of chromosome pairing. Chromosoma (Berl.) **8**, 380—395 (1957). — Histones of mitosis and meiosis in *Loxa flavicolis (Hemiptera)*. J. biophys. biochem. Cytol. **4**, 59—62 (1958).

Bonhag, P.: Histochemical studies of the ovarian nurse tissues and oocytes of the milkweed bug *(Oncopeltus fasciatus)*. J. Morph. **96**, 381—440 (1955). — Ovarian structure and vitellogenesis in insects. Ann. Rev. Entomol. **3**, 137—160 (1958).

Bowen, R. H.: Notes on the occurrence of abnormal mitoses in spermatogenesis. Biol. Bull. **43**, 184—203 (1922a). — Studies on insect spermatogenesis. IV. (Proc. Am. Acad. Arts Sci. **57**, 391—423 (1922b).

COOPER, K. W.: Cytogenetic analysis of major heterochromatic elements (especially Xh and Y) in *Drosophila melanogaster*, and the theory of "heterochromatin" Chromosoma (Berl.) **10**, 535—588 (1959).

MARTIN, B.: Temporary elimination of the autosomes from the meiotic spindle in a halyinid pentatomid. J. Morph. **92**, 207—239 (1953).

MONTGOMERY, T. H.: The spermatogenesis of *Pentatoma* up to the formation of the spermatid. Zool. Jb., Abt. Anat. u. Ontog. **12**, 1—88 (1898). — On the dimegalous sperm and chromosomal variation of *Euschistus*, with reference to chromosomal continuity. Arch. Zellforsch. **5**, 120—146 (1910).

NICKLAS, R. B.: Paper in preparation.

RUCKES, H.: Three new species of *Mecistorhinus* DALLAS *(Heteroptera, Pentatomidae)*. Amer. Mus. Novitates **1930**, 1—8 (1959a). — New genera and species of pentatomids from Panama and Costa Rica. Amer. Mus. Novitates **1939**, 1—18 (1959b).

SCHRADER, F.: Heteropycnosis and non-homologous association of chromosomes in *Edessa irrorata (Hemiptera Heteroptera)*. J. Morph. **69**, 587—607 (1941). — Regular occurrence of heteroploidy in a group of *Pentatomidae (Hemiptera)*. Biol. Bull. **88**, 63—70 (1945a). — The cytology of regular heteroploidy in the genus *Loxa (Pentatomidae-Hemiptera)*. J. Morph. **76**, 157—177 (1945b). — The elimination of chromosomes in the meiotic divisions of *Brachystethus rubromaculatus* DALLAS. Biol. Bull. **90**, 19—31 (1946a). — Autosomal elimination and preferential segregation in the harlequin lobe of certain *Discocephalini (Hemiptera)*. Biol. Bull. **90**, 265—290 (1946b).

—, and C. LEUCHTENBERGER: A cytochemical analysis of the functional interrelations of various cell structures in *Arvelius albopunctatus* (DE GEER). Exp. Cell Res. **1**, 421—452 (1950). — The cytology and chemical nature of some constituents of the developing sperm. Chromosoma (Berl.) **4**, 404—428 (1951).

SRIVASTAVA, M. D. L.: Compound sex chromosome mechanism and regularly occurring meiotic aberrations in the spermatogenesis of *Macropygium reticulare*. Cellule **58**, 252—274 (1957).

TROEDSSON, P. H.: The behavior of the compound sex chromosomes in the females of certain *Hemiptera*. J. Morph. **75**, 103—147 (1944).

WEBER, H.: Biologie der Hemipteren. Berlin: Springer 1930. — Lehrbuch der Entomologie. Jena: Gustav Fischer 1933.

WILSON, E. B.: Studies on chromosomes. VIII. J. exp. Zool. **13**, 345—449 (1912).

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Chromosoma (Berl.) 11, 129—145 (1960)

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THE INDUCTION OF NEW PUFFING PATTERNS BY TRANSPLANTATION OF SALIVARY GLAND NUCLEI INTO EGG CYTOPLASMA OF DROSOPHILA

By

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With 5 Figures in the Text

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I. Introduction

It has long been assumed that there is no uniformity of activity of the genes of an organism, either at different developmental stages or in different tissues. Support for this notion is given by recent detection of morphological changes undergone by certain segments of the giant chromosomes in salivary glands during development of Diptera as well as differences in giant chromosome morphology in different tissues (BEERMANN 1952a, b, BREUER and PAVAN 1953, 1955, MECHELKE 1953, BECKER 1959). In fact, the evidence points toward the hypothesis that the differences between tissues and the progress in development of an organism are brought about by the differential activation or suppression of different sets of genes (BEERMANN 1959). Progress in linking the observed morphological changes in the chromosomes to known genetic factors and in understanding the factor that control the onset, duration, and end of a specific activity of genes was seriously hampered by the fact that no genetic loci are known that affect the salivary gland and only very few tissues besides salivary glands contain chromosomes sufficiently polytene to be suitable for morphological studies.

In an effort to overcome this barrier, use was made of VON BORSTEL's discovery (1959) that, in the exudate of *Habrobracon* eggs, nuclear divisions can go on for at least a few hours if the exudate is covered with mineral oil to prevent evaporation. The present paper describes a method for transplanting salivary gland nuclei of *Drosophila* into egg cytoplasm of different developmental stages in an effort to induce differential chromosome puffing. Puffs are swellings in limited chromosome regions and are presumed to be signs of a high metabolic activity.

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II. Material and Technique

The routine experiment consisted in transplanting *Drosophila busckii* salivary gland nuclei into egg cytoplasm of *Drosophila melanogaster*. The *D. busckii* larvae came from a stock derived from a wild population collected in Princeton in 1952 and since then inbred and kindly supplied to me by Dr. J. KRIVSHENKO. The *D. melanogaster* eggs were obtained from a wild strain (Oregon-R). *D. busckii* could not be raised satisfactorily on the standard food used in this laboratory. Instead, they were grown on a medium of the following composition: water, 1000 ml; agar, 10 g; "Karo" white crystal syrup, 100 g; brewers yeast (dry), 50 g; corn meal, 85 g; flax seed meal, 6 g; Tegosept, 10 ml of a 10% solution Tegosept in 70% alcohol. The eggs from the *D. melanogaster* wild strain were collected by pressing them out of the uterus of mated females that had been put on fresh medium the day before. When they were transferred into a drop of oil their stage of development could be easily determined with transmitted light under a dissecting microscope.

Two stages were sorted out: (1) eggs before blastoderm formation, which still showed a homogeneous egg cytoplasm (the term egg cytoplasm refers in this paper to the whole content of the egg, including nuclei), i. e., before appearance of the irregular clouding that precedes blastoderm formation (henceforth called "pre-blastoderm eggs") and (2) eggs near the completion of blastoderm formation, when the clear zone of blastoderm reaches its maximum width (called "blastoderm eggs"). Sometimes the development of eggs was arrested before use by putting them into a refrigerator (4—5° C) for a period not exceeding 1 hour.

The transplantation was performed under halofluorocarbon oil. Halofluorocarbon polymers are manufactured by the Minnesota Mining and Manufacturing Company, Jersey City, New Jersey, under the trade name of "Kel-F" in various stages of polymerization. For our experiments "heavy oil" (No. 10) was used. The Kel-F polymer molecule consists of a carbon skeleton and two halogens, fluorine and chlorine. It has an extremely low moisture vapor transmission and has been shown by KOPAC (1955a, b) to be very inert with respect to living cytoplasm.

The nuclei were incubated on microscope slides. For good attachment of the cytoplasm to the glass surface, it was necessary that the slides be prewashed but *not* chemically cleaned, since the oil as well as the cytoplasm tend to spread on chemically cleaned slides.

The actual process of transplantation consisted in the following steps: (1) Five eggs of a given stage were placed on a microslide and covered with a drop of halofluorocarbon oil. Four of them were pinched at a pole, their contents squeezed out and brought into contact so as to form one droplet on the surface of the slide. It was essential that this droplet remain more or less spherical so that the transplanted nuclei were surrounded by ample cytoplasm. The content of the fifth egg was similarly made into a small droplet under the same drop of oil. (2) The egg cytoplasm was adjusted to the tonicity required for survival of salivary gland nuclei by adding $4.2 \times 10\,\mu$ of distilled water (as determined by the diameter of a water sphere in the oil with an ocular micrometer). The water was introduced with a micropipette, as described by KROEGER (1959), into the large droplet and immediately stirred in thoroughly with a glass needle. The stirring process was

continued for about one-half minute, during which time the clear blobs of blastoderm (in "blastoderm eggs") became homogenized with the other egg materials. (3) A larva of *D. busckii* war dissected in the modified Ringer's solution described by EPHRUSSI and BEADLE (1936) on a corner of the slide used in steps 1 and 2, and one of the salivary glands [the other one was used as a control, see (4)] was placed in the smaller droplet of cytoplasm. There it was dissected with glass needles that had been drawn out to very fine tips. For the actual dissection, needles were used that had been treated with silicon oil to avoid adherence of cytoplasm. By use of non siliconed needles the isolated nuclei in the small droplet were pushed through the oil into the large droplet. Then the small droplet and the remains of the gland were removed. (4) The other salivary gland was used as a control. It was transferred either into a small droplet of the modified Ringer's solution in a depression slide covered by a cover slip and sealed with paraffin oil or it was brought as a whole into a droplet made, as described in (1), of four eggs of the same age as the eggs in which the other gland was incubated. After 3 hours they were processed as in (5). In the beginning, controls were kept alternatively in Ringer's solution and in egg cytoplasm. Since no conspicuous difference appeared (see p. 139), only Ringer's solution was used in later experiments. (5) As a standard procedure the nuclei were left in the cytoplasm for 3 hours. Then, with a tuberculin syringe, a small drop of lactic-aceto-orcein (2% orcein in equal parts of glacial acetic acid and 85% lactic acid) was delivered into the oil drop and brought into contact with the cytoplasm so as to cover it. After 10 minutes, the oil was removed by placing the slide vertically into a staining dish with acetone for 3 minutes. Subsequently the slide was transferred into distilled water. Since the fixed cytoplasm adheres firmly to the slide during this procedure, no losses occurred at this stage. After 10 or more minutes (an overnight stay in distilled water did not visibly damage the preparation), the slide was removed from the water, dried with blotting paper except for a tiny amount of liquid aorund the fixed drop. The cytoplasm droplet was then covered with another drop of lactic-aceto-orcein for 5 minutes and then squashed.

Dissection of the nuclei is facilitated by the use of a special type of lighting. The isolated nuclei stand out clearly from the cytoplasm when a mixture of direct and transmitted light is used at a rather steep angle to the surface of the preparation.

Presence or absence of structural modifications was best determined in overstained preparations, that is, in preparations at least one week old.

III. Results

1. General conditions affecting the result of transplantations

A number of factors were important for survival of the transplanted nuclei.

a) Tonicity. If nuclei are transplanted without previous adjustment of the tonicity of the egg cytoplasm, a typical syndrome occurs within 30 to 40 minutes: the chromosomes become thinner and begin to form vacuoles in the bands but not in the interbands (the same observation was made in nuclei from whole control glands kept in hypertonic saline solution). Soon the vacuoles expand the bands so that they can no longer be differentiated from the interbands — the staining becomes diffuse all along the chromosome. Excess water, on the other hand, causes a lateral swelling of the chromosomes, making the otherwise

solid, heavily staining bands appear to consist of dots. The characteristics of individual bands were thereby sifficiently altered to prevent their identification.

b) The plasma coat. A thin coating of plasma during transplantation was essential to survival of the nuclei. If the salivary gland cytoplasm is of the right viscosity, the coating stays around the nucleus without difficulty. This viscosity could be achieved by a numer of different conditions slightly adverse to larval growth. Dryness of the food, excessive amounts of yeast, or above-optimum temperature (about 27°C) had this effect but in a very irregular manner. Overpopulation and high humidity did not have the desired effect; in fact, they made the cytoplasm particularly liquid so that the naked nucleus without any adhering cytoplasm popped out as soon as the cell was ruptured. None of these nuclei ever survived. The plasma coat seemed to be the most decisive factor (together with the age of the donors) in survival of the nucleus and was also the hardest to control. The usual procedure was to test a number of cultures exposed to the conditions mentioned and to pick one that gave good survival. This culture was subsequently used for several days.

c) Age of the donor larvae. The age range of donor larvae that would yield nuclei suitable for transplantation was rather narrow. Beyond the middle of the last instar the salivary gland of *D. busckii* is rather uniform in thickness all along its length and is completely transparent. Toward the end of the larval life the distal portion begins to thicken and takes on an opaque appearance. The opaqueness proceeds proximally and the nuclei become swollen. The surface of the gland, which in the beginning of this process was raspberry-like (the individual cells bulged), finally becomes smooth and the opaqueness becomes irregular. The best time for obtaining nuclei for transplantation was after the onset of the opaqueness and the thickening of the distal part of the gland but *not later than the time at which this process reaches the middle of the gland.* This corresponds to the point in development just before the larvae leave the medium to pupate but are still actively feeding. The anterior spiracles, which can easily be everted by applying pressure on the body of the larva, are at this time still completely uncolored.

An interesting phenomenon was observed during incubation of nuclei in blastoderm egg cytoplasm. The pressed-out contents of blastoderm eggs consisted of blobs of clear plasm in the opaque background of the rest of the egg contents. As mentioned in (2) of Material and Technique, the subsequent stirring process disperses these blobs completely. After a while, however, the clear plasm begins to reaggregate into islands, the centers of which are occupied by the implanted nuclei. Soon the nuclei become completely ensheathed by a clear zone of cytoplasm. The possible influence of this phenomenon on the results will be discussed later.

2. The normal puffing pattern

Maps of the salivary glands of *D. busckii* have been presented by SIROTINA (1938), but they are very schematic and do not permit recognition of individuals bands. The locations given in the present paper correspond, therefore, only approximately to the regions in SIROTINA's maps. KRIVSHENKO's terminology was followed in that the elements A, B, C, and D are called IIL, IIR, IIIL, and IIIR, respectively. For the X chromosome, KRIVSHENKO (1955) has published a detailed map that permits exact correspondence of observed modifications with a particular region on the map.

The salivary gland chromosomes of *D. busckii* offer a number of advantages over other species in the recognition of specific areas. Foremost among them is the absence of a chromocentral mass that masks the connections between chromosome arms and impairs the preparation of smears with well-separated chromosomes. Furthermore, the chromosomes of *D. busckii* are easily stained and show a very distinct banding pattern.

The general appearance of transplanted chromosomes was often considerably changed. The descriptions in this paper are therefore restricted to only a few regions studied in detail. These regions were either near a landmark or were otherwise easily recognizable and contained particularly conspicuous puffs.

The spectrum of puffing patterns changes continuously during development. Puffing patterns *in situ* were investigated at many points during larval development. Two were chosen as being representative of the normal changes that occur around the period of puparium formation: the time shortly before puparium formation ("prepuparium pattern") and approximately 1 to 2 hours after pupation ("postpuparium pattern"). At these two stages the puffs described seemed to be particularly pronounced; no additional puffing was observed in the regions studied intensively between these stages. The same was true for a period after the stage taken as representative for the postpuparium pattern.

The prepuparium pattern corresponds to the condition of the nuclei at the time of transplantation, and the postpuparium pattern roughly corresponds (as will be shown in the discussion of the controls for the transplanted nuclei) to the pattern the chromosomes would have reached had they remained *in situ* during the standard incubation time.

On the basis of these restrictions, the puffing patterns can be described as follows:

a) The prepuparium pattern. In the X chromosome, section 3 (Fig. 1 a) contains a large puff characterized by a band of rather heavy dots through the widest portion. These dots later become less distinct to result in a more uniform appearance of the puff. Section 6 contains a much smaller puff bordered on both sides by particularly densely

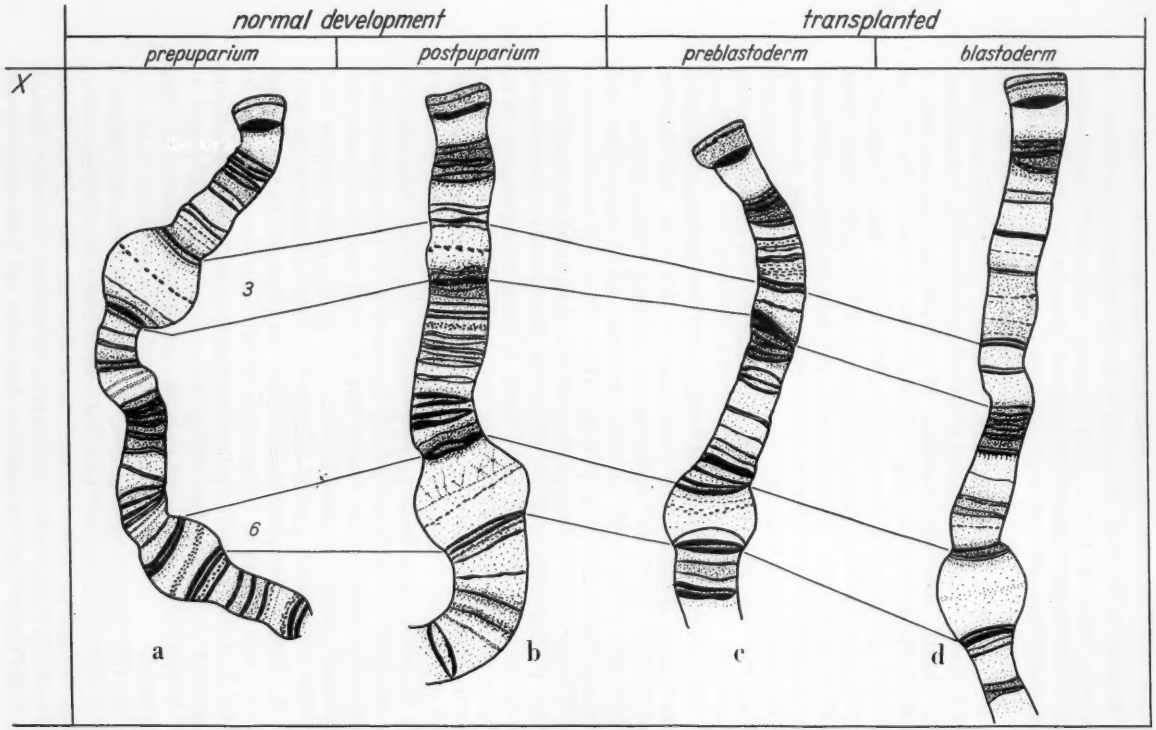

Fig. 1 a—d

Fig. 1—5. Comparison of regions of salivary gland chromosomes of *Drosophila busckii* before puparium formation (a), after puparium formation (b), after incubation for 3 hours in egg cytoplasm from eggs before blastoderm formation (c), and near the completion of blastoderm formation (d). Orientation of the chromosomes with the distal end toward the top of the page. The chromosomes in 1 c, 3 b, 4 d, and 5 b had sharp bends in the stained preparations but have been drawn straight to facilitate comparisons. All drawings were made by *camera lucida*

stained bands. This puff is at this time rather inconspicuous. Chromosome IIR contains at its proximal end a region that easily breaks between sections 21 and 22 (this occurred in at least half of all preparations), which results in a fragment having a characteristic shape and banding pattern. The proximal two-thirds of the fragment is unusually heavily staining (23—25) and is adjoined by a celar zone and a few heavy bands next to the breaking point (22). This piece, particularly when broken of, is among the easiest to recognize in the whole chromosome set. Its clear zone has never been observed to puff in normal development. Chromosome IIL contains in the middle of its distal half (section 8) the largest puff that can be observed in normal development (Fig. 3a). Chromosome IIIR shows, distal to a very heavily stained discoidal band in section 8, a region that is sometimes slightly puffed, this structural modification being irregular and not pronounced (Fig. 4a). Chromosome IIIL contains in section 6 a small but clear and very stable puff. Since the particular structure of section 4 makes it an obvious landmark, the puff in section 6 can be easily recognized (see Fig. 5a). No puff can be seen at this time in section 8. The presence or absence of puffs at this time of development is summed up in the following table.

Table. *Puffing in salivary gland chromosomes*

Presence (+), absence (—) of specific puffs, and impossibility to recognize the respective region (0) in salivary gland chromosomes after incubation in egg cytoplasm of preblastoderm (a) of blastoderm eggs (b) and presence or absence of these puffs in normal development (c, d).

	X/3			X/6			II R/22			II L/8			III R/8			III L/6			III L/8		
	+	—	0	+	—	0	+	—	0	+	—	0	+	—	0	+	—	0	+	—	0
Nuclei transplanted into:																					
(a) Preblastoderm eggs	0	26	46	14	4	54	56	2	14	0	30	43	22	16	35	48	3	21	0	30	42
(b) Blastoderm eggs	0	15	27	8	0	36	1	25	19	0	25	30	23	1	24	15	1	30	0	10	36
Nuclei *in vivo:*																					
(c) Prepuparium pattern	+			+			—			+			(+)[1]			+			—		
(d) Postpuparium pattern. . . .	(—)[2]			+			—			(—)[2]			+			+			+		

[1] Before puparium formation only the distal puff developed and was small (see Fig. 4a). After puparium formation and in incubated nuclei a pronounced double puff appeared.

[2] Sometimes slight remnants of the puff were observed (see Fig. 3b).

b) The postpuparium pattern. In the X chromosome (Fig. 1b), the large puff in section 3 has disappeared; sometimes slight remnants of it can still be seen. The puff in section 8, however, is present. In

preparations but have been drawn straight to facilitate comparisons. All drawings were made by *camera lucida*

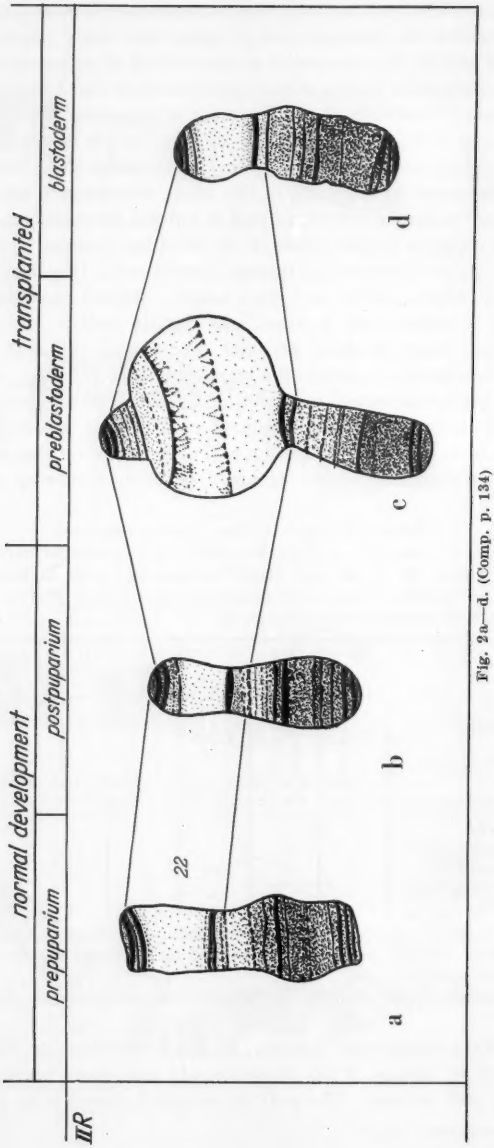

Fig. 2a—d. (Comp. p. 134)

IIR the characteristic piece 22—25 is unchanged and shows no puffing (Fig. 2b). The large puff in IIL/8 has usually completely disappeared (Fig. 3b); only very seldom are remnants of the puff found. In IIIR, in the formerly slightly puffed region in section 8, a pronounced and clear double puff has developed at each side of the heavily stained band mentioned (23—25) above (Fig. 4b). In IIIL the puff in section 6 has persisted, and another rather large one has appeared in section 8.

3. The puffing pattern induced by preblastoderm egg cytoplasm

In scoring a nucleus after incubation in cytoplasm, regions of interest were located and scored for the presence (+) or absence (—) of a puff; if it was not possible to recognize a particular region that nucleus was scored "0" for the region (Table). Only nuclei in which at least one of the investigated regions could be identified was recorded. As can be seen in Table, the number of nuclei scored as +, — , and 0 do not always correspond to the total number of nuclei studied for all chromosomes. This is because the nuclear origin of a fragment occasionally could not be determined or the chromosomes of two nuclei were intermingled.

A bias may be expected in the results because certain regions can be more easily identified when they contain a characteristic puff; this would lead to an excess of + counts for that region. Only region IIIR, with its double puff, could be subject to this bias. The rest of the regions are readily identified; sections 22 to 25 in IIL, because of the characteristic appearance of the whole region under all experimental conditions; the region investigated in IIIL, because of the peculiar structure in its section 4. IIL/8 was observed in the puffed conditions only in normal development (Table); the X/8 puff was observed both *in situ* and in transplanted nuclei.

After an incubation of nuclei for 3 hours in preblastoderm egg cytoplasm the chromosomes appeared as follows (Table, line a): In the X chromosome the large puff in section 3 has completely disappeared in all cases; the one in section 6 has persisted in most cases (Fig. 1c). In IIR/22 a puff has appeared that was never encountered in normal development and that sometimes exceeds in size all other puffs seen in normal development or in transplanted nuclei (Fig. 2c). This puff is also very consistent in its appearance (Table, line a). In IIL/8 the puff that is very striking in the prepuparium pattern is absent in all cases (Fig. 3c and Table, line a). In IIIR/8 there is a pronounced double puff; its appearance is irregular however, and only a fully developed double puff on both sides of the heavily stained band was recorded as + to avoid confusing this appearance with the weak development of a puff distal to that band in the prepuparium pattern (p. 135). In IIIL the puff in 6 has persisted but none has appeared in section 8 (Fig. 5c and Table, line a).

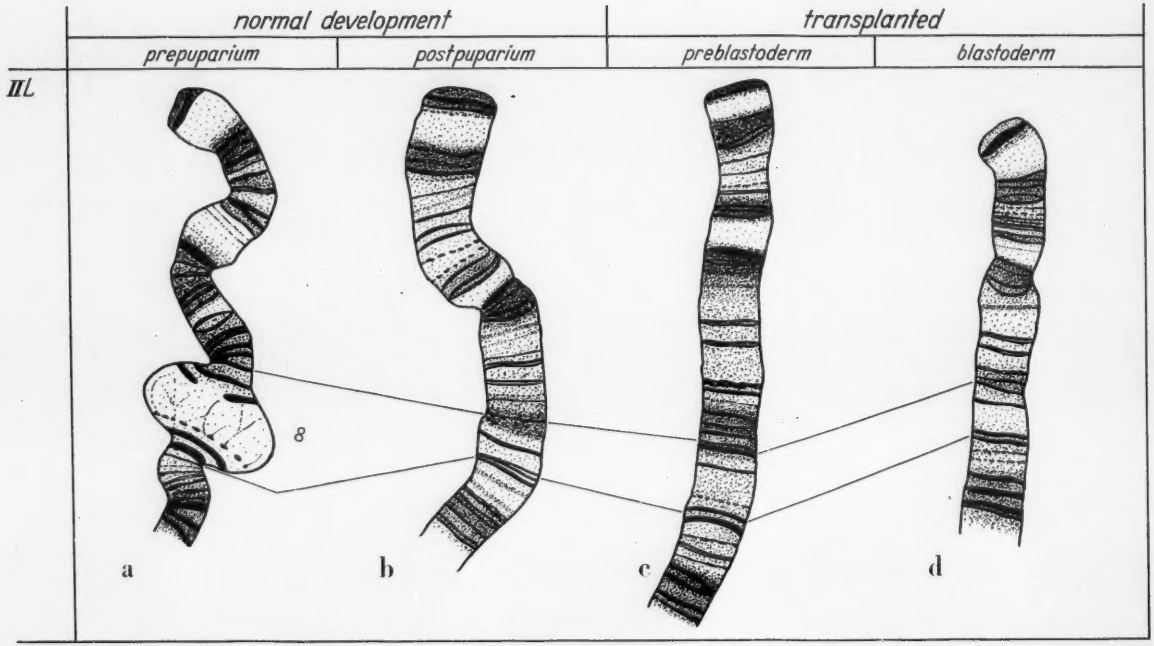

Fig. 3a—d. (Comp. p. 134)

4. The puffing pattern induced by blastoderm egg cytoplasm

After incubation for 3 hours in blastoderm egg cytoplasm (Table, line b), the X chromosome did not contain a puff in section 3 (Fig. 1d) but did show a puff in 6. Almost never (Table, line b) could a puff be induced in section 22 of IIR (Fig. 2d). IIL/8 appeared unpuffed in all cases (Fig. 3d). The double puff in IIIR/8 is now very pronounced (Fig. 4d); in IIIL the puff in 6 persists but no puff is found in 8 (Fig. 5d).

The conditions in normal development and after incubation can be summed up as follows for the single puffs: The puff in X/3 is present in prepuparium, disappears in postpuparium chromosomes, and is never found after incubation in either kind of cytoplasm. The puff in X/6 persists through the stages of puffing patterns described and also through incubation in both types of cytoplasm. In section 22 of IIR, no puff is ever observed in normal development; preblastoderm egg cytoplasm induces a very large puff in IIR/22; blastoderm cytoplasm has no effect. The puff in section 8 of IIL disappears during normal development from the prepuparium to the postpuparium stage. It also is absent after incubation in egg cytoplasm of either stage. The double puff in IIIR/8 is small and inconspicuous in prepuparium chromosomes and becomes pronounced in the postpuparium stage; the same effect is achieved in the majority of the nuclei by incubation in either kind of cytoplasm. In IIIL the puff in section 6 was encountered in all investigated stages of normal development as well as during incubation in either cytoplasm. Only in the postpuparium pattern of normal development was a puff recorded in section 8.

5. The controls

The two types of controls (incubation of whole glands in egg cytoplasm or in Ringer's solution for 3 hours) yielded about equal results. BECKER (1959) has demonstrated that, after larvae of *D. melanogaster* have reached a certain age, their polytene chromosomes exhibit essentially the same sequence of puffing whether the glands are kept *in vivo* or *in vitro*. The same was true, in our experiments, for *D. busckii*. The pattern developed, but the sizes of the puffs were rather irregular (more so, in fact, than in the incubated nuclei from the other gland); that is, some preparations showed only the beginnings of the change toward a new puffing pattern, and usually the puffs were rather small. The majority, however, developed a pattern that must be regarded as identical with the postpuparium pattern described. The control nuclei developed a puff in region IIIL/8 that experience has shown to be unique to the postpuparium pattern. Particular care was taken in examining region 22—25 of IIR, where no puffing could ever be observed

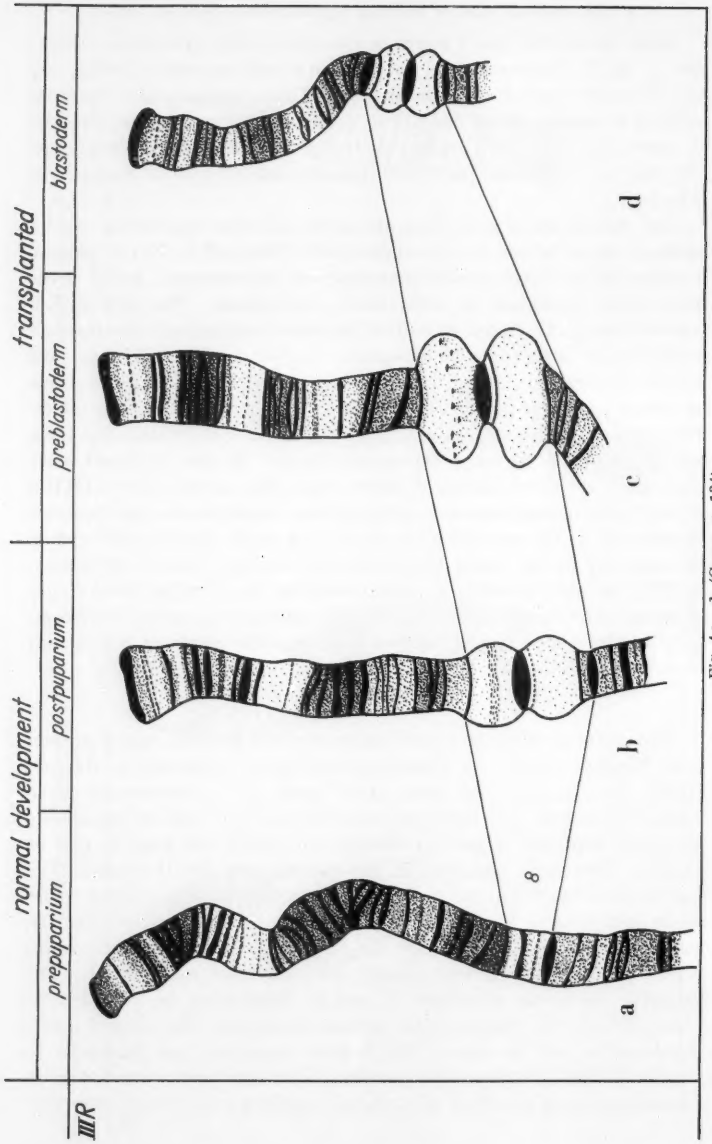

Fig. 4a—d. (Comp. p. 134)

IV. Discussion

First, can the changes induced by the egg cytoplasm in isolated nuclei be regarded as physiological, i. e., as nucleocytoplasmic interactions comparable to the process occurring in normal development? In view of the differential response of the different sections to the new environment (disappearance of X/6 and IIL/8, persistence of IIIL/6, appearance of IIIR/8 and IIR/22), it seems rather unlikely that these changes represent only a degeneration syndrome. In degeneration we might expect a disappearance of all puffs or the development of puffing at many places along the chromosomes rather than such specific responses.

The second question is whether the transplanted nuclei had already been induced to form the postpuparium pattern before transplantation, i. e., to proceed in their normal development irrespective of the new environment, as could easily be the case since the nuclei carry a coat of their original cytoplasm. The appearance of IIR/22 in preblastoderm plasm and the nonappearance of IIIL/8 differentiate the induced pattern from the postpuparium pattern. Some puffs, however, may have been induced before transplantation and others not, since puffs seem to appear independently and the puffing pattern is probably only the result of the independent action of certain bands at certain times. In normal development, of course, the physiological activity of the genes must be coordinated by way of the cytoplasm.

Third, is the induced puff in IIR/22 a phenomenon comparable to the one observed by BECKER (1959)? When salivary glands of *D. melanogaster* of a specific age were kept in Ringer's solution (of a different composition than the one used in our experiments), he observed an abnormal puff, i. e., one that had not been observed in normal development. First it has to be said that, in our experiments, IIR/22 could not be induced by incubating whole glands in Ringer's solution. It should be noted that puff IIR/22 could not be induced even by incubation of whole glands in egg cytoplasm. Since preblastoderm cytoplasm could induce IIR/22 in isolated nuclei, whereas blastoderm plasm could not, this puff must be a response to a rather specific factor that is present in preblastoderm but absent in blastoderm cytoplasm. A comparison with BECKER'S abnormal puff in Ringer's solution does not seem, therefore, an apt one. It is possible, however, that it is not the presence or absence of a chemical factor but the distribution of the factor in the egg that is responsible for the differential reaction in the two stages of embryonic development. The factor could reside, for instance, in the yolk of the egg and therefore be absent in the clear plasm of the blastoderm stage. The aggregation of this clear plasm around the nuclei implanted into the content of blastoderm eggs could have shielded the nuclei from the influence of this factor. Similar conditions, exposure

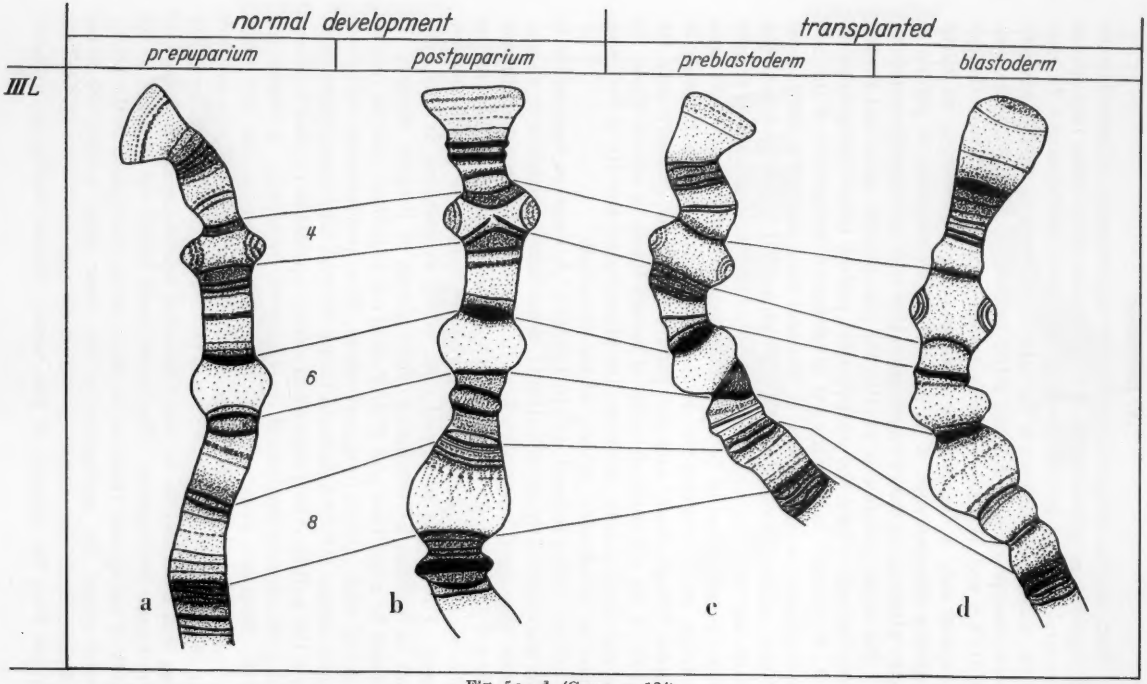

Fig. 5a—d. (Comp. p. 134)

of nuclei to yolk or shielding of them from its influence by protein, may very well exist during normal development to bring about changes in the reactivity of the nuclei.

BEERMANN (1959) reviewed the results pertaining to an interpretation of the structural modifications in giant chromosomes as signs of a differential physiological activity of genes. He pointed out that the low number of loci with visible modifications does not necessarily mean that all the other loci have to be regarded as completely inactive during the period of observation. Systems in which large amounts of specific products are manufactured (as in gland cells, for instance) seem to be the most promising ones to use in a search for factors capable of inducing visible reactions at the site of the genetic information. Such a system is early embryonic development because a number of metabolic processes probably have high rates of turnover (as, for instance, synthesis of proteins) that change with progression of development, thereby changing the pattern of activity along the chromosomes. Our present knowledge on control mechanisms of cellular physiology does not permit us to state whether two groups of genes exist, one of which is concerned with the execution of metabolic processes whose stimulation, regulation, and suppression is controlled by the second group of genes. In any case, it remains speculative whether the puffs induced in transplantation really correspond to loci that are responsible for processes characteristic of early embryonic development and whether the change in the inducing capability from preblastoderm to blastoderm egg cytoplasm reflects the change of activity patterns that occur during development. This problem will have to await an identification of the chemical processes with which the puffed regions are concerned, an undertaking that seems feasible with the method described in this paper.

If we adopt the concept of differential gene activation as a working hypothesis, a number of classes of processes can be expected to express themselves as different puffing patterns in different stages of development and in different environments. Some puffs might represent metabolic processes so basic that they would be expected to persist all through development and in all environments, such as respiration or protein synthesis. X/6, IIIL/6, and possibly IIIR/8 (about the last one see p. 137) might be examples of such puffs. Another class might represent specific processes, such as the production of saliva, and therefore would not be expected to find expression during exposure to egg cytoplasm. Therefore, those puffs that are present in prepuparium nuclei and disappear after puparium formation (when production of saliva would seem useless) do not appear during incubation in egg cytoplasm (X/3 and IIL/8). Another class of processes might represent loci active only at certain stages of embryonic development; genes involved in the

utilization of yolk, for example, would not be expected to be active in salivary gland nuclei. This could be the case for II R/22.

It is obvious that the technique described in this paper invites a number of further investigations and puts interesting questions within the reach of experimental investigation. The attack on the chemistry of gene activation has already been mentioned. By introducing salivary gland chromosomes of different dipteran species into the same environment, it should be possible to determine by physiological criteria the "homolgy" of certain loci in the sense that they are involved in similar or identical functions. Many combinations of donor nuclei and cytoplasm may be envisaged, such as transplantation of nuclei into eggs known to contain early embryonic lethal alleles, into immature (ovarial), and into nullo-X eggs.

Zusammenfassung

1. Speicheldrüsen aus erwachsenen Larven von *Drosophila busckii* wurden isoliert und in Cytoplasma von Eiern verschiedener Alterstadien von *Drosophila melanogaster* transplantiert.

2. Die Normalentwicklung des Puff-Musters sowie die Entwicklung nach 3stündigem Aufenthalt im Eicytoplasma wird beschrieben. Die Puffs in X/3 und II L/8 sind in Kernen aus Larven vor der Puparium-bildung vorhanden und verschwinden danach. Sie fehlen nach Aufenthalt im Eiplasma. Die Puffs X/6 und III L/6 sind sowohl in der Normalentwicklung wie nach Transplantation in Eicytoplasma stets vorhanden. Das gleiche gilt für III R/8, doch ist dieser Doppelpuff vor der Puparium-bildung nur selten und teilweise entwickelt. Puff II R/22 erscheint nie in der Normalentwicklung, wird durch Eicytoplasma aus Eiern vor der Blastodermbildung induziert, nicht aber von Cytoplasma aus Eiern auf dem Höhepunkt des Blastodermstadiums. Puff III L/8 erscheint nur nach der Puparium-bildung in der Normalentwicklung; er fehlt in transplantierten Kernen.

3. Als Kontrolle wird die zweite Speicheldrüse der zur Gewinnung von Kernen benutzten Larven für 3 Std als Ganzes in Ringer-Lösung bzw. Eicytoplasma gehalten. Diese Kontrolldrüsen entwickeln sich im wesentlichen zu dem Stadium weiter, das für Larven nach der Puparium-bildung typisch ist. Dieser Entwicklungsprozeß ist unregelmäßig und oft unvollständig.

References

BECKER, H. J.: Die Puffs der Speicheldrüsenchromosomen von *Drosophila melanogaster*. 1. Beobachtungen zum Verhalten des Puffmusters im Normalstamm und bei zwei Mutanten, *giant* und *lethal-giant-larvae*. Chromosoma (Berl.) **10**, 654—678 (1959).

BEERMANN, W.: Chromomerenkonstanz und spezifische Modifikationen der Chromosomenstruktur in der Entwicklung und Organdifferenzierung von *Chironomus tentans*. Chromosoma (Berl.) **5**, 139—198 (1952a). — Chromosomen-

struktur und Zelldifferenzierung in der Speicheldrüse von *Trichocladius vitripennis*. Z. Naturforsch. **7b**, 237—242 (1952b). — Chromosomal differentiation in insects. In: Developmental Cytology, edit. D. RUDNICK, p. 83—103. New York: Ronald Press Co. 1959.

BORSTEL, R. C. v.: Free nuclear division in exudates from eggs. Fed. Proc. **18**, 164 (1959).

BREUER, M. E., and C. PAVAN: Salivary chromosomes and differentiation. Proc. 9th Internat. Congr. Genet., Part II. Caryologia (Firenze) **6**, Suppl., 778 (1954). Behavior of polytene chromosomes of *Rynchosciara angelae* at different stages of larval development. Chromosoma (Berl.) **7**, 371—386 (1955).

EPHRUSSI, B., and G. W. BEADLE: A technique of transplantation for *Drosophila*. Amer. Naturalist **70**, 218—225 (1936).

KOPAC, M. J.: Recent development in cellular micrurgy. Trans N. Y. Acad. Sci., Ser. II **17**, 257—265 (1955a). — Cytochemistry: its problems and instrumentation. Trans. N. Y. Acad. Sci., Ser. II **18**, 22—29 (1955b).

KRIVSHENKO, J. D.: Comparative genetic study of *Drosophila busckii* and *Drosophila melanogaster*. Mem. Genet., Acad. Sci. Ukr. SSR. **3**, 15—89 (1939). — A cytogenetic study of the X chromosome of *Drosophila busckii* and its relation to phylogeny. Proc. nat. Acad. Sci. (Wash.) **41**, 1071—1079 (1955).

KROEGER, H.: Determinationsmosaike aus kombiniert implantierten Imaginalscheiben von *Ephestia kuehniella* ZELLER. Wilhelm Roux' Arch. Entwickl.-Mech. Org. **151**, 113—135 (1959).

MECHELKE, F.: Reversible Strukturmodifikationen der Speicheldrüsenchromosomen von *Acricotopus lucidus*. Chromosoma (Berl.) **5**, 511—543 (1953).

SIROTINA, M. I.: A cytological study of *Drosophila busckii*. Mem. Genet., Acad. Sci. Ukr. SSR. **2**, 61—90 (1938).

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Chromosoma (Berl.) 11, 146—166 (1960)

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THE NATURE OF THE INFLUENCE OF X-TRANSLOCATIONS ON SEX OF PROGENY IN SCIARA COPROPHILA

By

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With 11 Figures in the Text

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I. Introduction and Background

A conspicuous differentiation of nuclei occurs in the genus *Sciara* as specific chromosomes are eliminated from particular cells during the course of embryonic development. Outstanding in this elaborate chromosome history is the behavior of the sex chromosome. There are three sex chromosomes in each zygote. After elimination, the somatic cells of the females retain two and the somatic cells of the males only one. All three remain in early germ cells, but later one is eliminated so that the gonads of both sexes contain two. Mature sperms have two sex chromosomes while the mature eggs contain only one. Our knowledge of this remarkable situation is based on the extensive genetic and cytological studies of Professor C. W. METZ and his associates (for reviews, see METZ 1938, BERRY 1941).

In an attempt to analyze the mechanism which controls the elimination of the sex chromosome during embryonic development and its unusual pattern of behavior during spermatogenesis, reciprocal translocations were induced between X and the autosomes (CROUSE 1943). Two different species, *S. reynoldsi* METZ and *S. coprophila* LINTNER, were used. *S. coprophila* is a species in which normally all or nearly all eggs laid by an individual female develop either into sons or into duaghters. This mode of reproduction has been termed "monogenic". In the strains of *S. coprophila* used in these studies individual females normally produced offspring exclusively of one sex. All the translocations involving the sex chromosome in this material, however, were found to induce a conspicuous alteration in the monogenic condition. Among the progeny of female-producing mothers, sterile exceptional sons were found; and among the progeny of male-producing mothers exceptional daughters occurred. Moreover, the frequency of the exceptional sons

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and daughters seemed to vary in a characteristic manner from one translocation to the other. Two hypotheses were suggested to account for the occurrence of the exceptional sex types: either they arose from certain kinds of non-disjunctional eggs or they were to be attributed to a genetic change induced on the rearranged X chromosome. The studies to be presented in the present paper are a continuation of the analysis and they point to the first hypothesis as the correct one.

Because the normal chromosome history of *Sciara* may be unfamiliar to some readers, I shall review very briefly those details which are essential to an understanding of the experiments. It should be realized at the outset that the chromosome eliminations encountered in *Sciara* fall into two main categories: those involving the sex chromosome and those which involve the large, deeply-staining "limited" chromosomes. The "limited" chromosomes are entirely absent in three known species of *Sciara*. In species such as *S. coprophila*, where they do occur, they play no known role in sex determination; they contain no known *bona fide* genetic loci; and, with their elimination from the embryonic somatic nuclei at the fifth cleavage division, they become restricted (hence, "limited") to the germ line. These chromosomes will be ignored throughout the remainder of this paper.

Stated very briefly, the pattern of inheritance of sex chromosomes in *S. coprophila* is as follows: instead of transmitting to the zygote a single sex chromosome, as does the egg, the sperm transmits two identical (sister halves) X's. Then, depending on the particular cells in question, an elimination of one or both paternal X's takes place during the course of embryonic development. The details as to the time of the elimination are not essential to this discussion. Suffice it to say, that in the production of female embryos only one paternal X is eliminated from both the soma and germ line, whereas in the production of male embryos both paternal X's are lost from the soma and only one from the germ line. Thus, the sex chromosome condition may be represented symbolically as XX for the germ line of both sexes, XX for the female soma, and XO for the male soma. In female-producing females, as will be noted below, one X chromosome differs genetically from the other.

Because the germ cell condition in the male is XX, one would expect, by orthodox standards, all of the sperm to include an X chromosome and a set of autosomes; but this is not the case. Both spermatocyte divisions are unusual. At the first division there is a selective segregation of maternal from paternal homologues. The paternal ones are cast away in a bud and the maternal ones are included in the secondary spermatocyte. The second meiotic division is equational for the autosomes, but both halves of the sex chromosome move to the same pole. Thus, since $n = 4$, three autosomes and two identical X's arrive at one pole; this set becomes included in the sperm nucleus. At the opposite pole a bud, containing three autosomes, forms and degenerates.

In this species, *S. coprophila*, the occurrence of the male or female type of sex chromosome elimination is controlled by the genetic constitution of the mother. The sex chromosome may be X-prime (X′) or X in genetic quality. Two kinds of females occur, XX′ and XX. The former are female-producers, the latter are

male-producers. Males are XX. A diagram is shown in Fig. 1 which illustrates the inheritance of sex chromosomes and the control of sex of progeny. In this diagram the X′ chromosome bears the dominant wing factor, *Wavy*, while the X chromosome bears its normal wild-type allele. The top portion of the diagram shows the two kinds of eggs, X′-bearing and X-bearing, formed by *Wavy* females and the daughters which result following fertilization; both the somatic and germ line complement

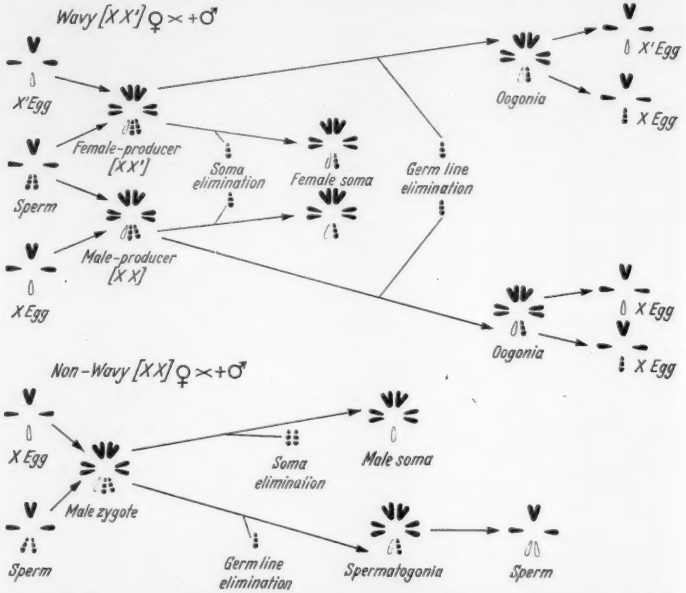


Fig. 1. Diagram of the normal chromosome history in monogenic *S. coprophila*; the X′ chromosome is carrying the dominant marker, *Wavy*, and the X chromosome the wild-type allele. The "limited" chromosomes have been omitted Above: *Wavy* (XX′) female bred so wildtype male. Below: Non-*Wavy* (XX) female bred to wildtype male

of the daughters are indicated. The lower portion of the diagram shows the single egg type formed by the *non-Wavy* or wild-type females and the sons which they produce; here, likewise, are indicated the somatic and germ line complements.

For five different translocations involving the X, cytological study was made (CROUSE 1943) of the testes of males which had inherited the rearranged chromosomes from their mother (only maternal homologues enter the seondary spermatocytes, the paternal ones being cast away in a bud). In each case only one of the rearranged chromosomes displayed behavior at the second spermatocyte division typical of the sex chromosome; i. e., both halves of it went to the pole and eventually became included in the sperm nucleus. The other rearranged chromosome

behaved like an autosome and underwent equational division. In three cases the rearranged chromosomes could be individually identified at metaphase and anaphase; and in these cases the chromosome which displayed behavior typical of the X was composed of a proximal segment of the X, including the X centromere, and a distal autosomal segment. The chromosome which behaved like an autosome possessed a proximal autosomal segment and a distal portion of the X.

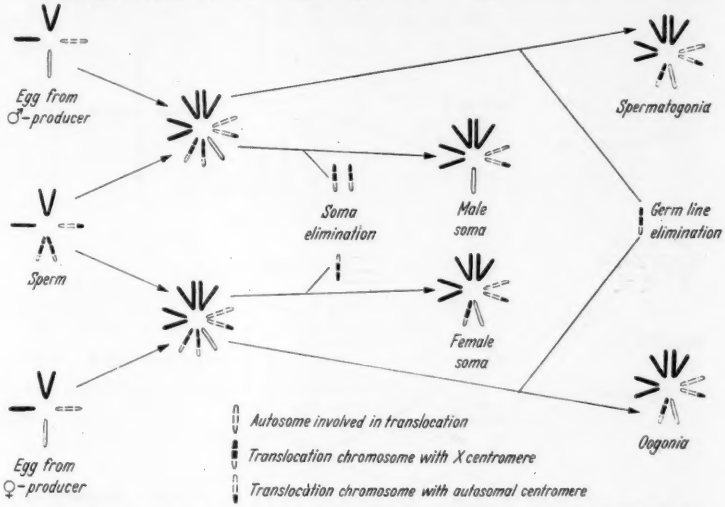


Fig. 2. Diagram of the chromosome history when an X-translocation is inherited from the father (sperm)

The diagram in Fig. 2 is based on the knowledge gained from the 1943 studies. It shows sperm which carry a reciprocal translocation between X and one of the rod-shaped autosomes. Two kinds of fertilization are indicated; eggs laid by female-producers are at the bottom of the diagram and eggs laid by male-producers are at the top. The chromosome constitution of the zygotes and the chromosome eliminations which ensue are also shown. The somatic chromosome complement of the sons differs from that of the typical *Sciara* males which have three pairs of autosomes and a maternal X. Instead of a paternally derived autosome, there is the translocation chromosome composed of autosomal material proximally and X material distally. *Actually this unbalanced somatic configuration has never been observed, because male embryos fail to hatch out of the eggs.* Presumably they cannot sustain such chromosomal unbalance. The daughters, on the other hand, are viable and fertile.

Their chromosome complement, somatic and germinal, is balanced and heterozygous for the translocation. In the diagram in Fig. 2, the genetic quality of the sex chromosomes is not shown. If it were, two kinds of eggs, X′ and X, would be indicated for the female-producers. Then, upon fertilization, half of the daughters would receive X′ and half the X. The chromosome configuration in both types, of course, is identical to

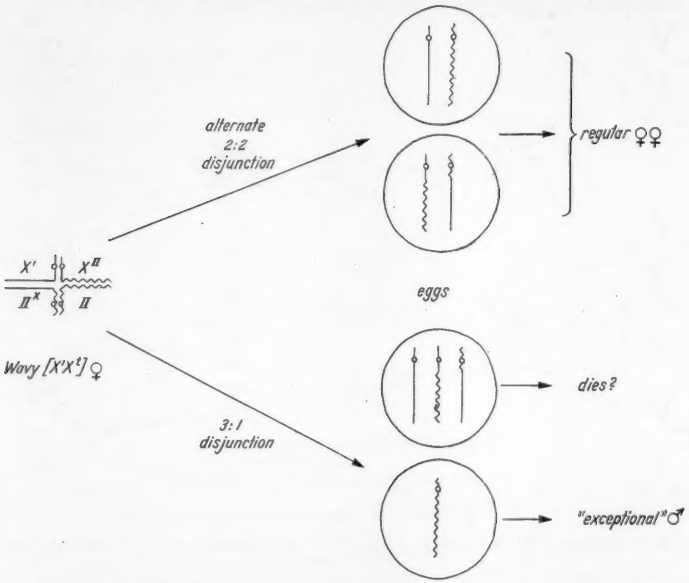


Fig. 3. Diagram of the egg types formed by female-producers heterozygous for PTl. *Wavy* is present on the normal X′ chromosome and *swollen* on the translocated X

that shown in the diagram. Using X^t as a symbol for the translocation, the two types of daughters could be represented $X'X^t$ and XX^t; the former should in turn be female-producers and the latter male-producers (the rearranged sex chromosome is an X).

As stated previously, it was in an effort to account for the exceptional sons and the exceptional daughters among the progeny of $X'X^t$ and XX^t females respectively, that the experiments reported in this paper were undertaken. For the translocation studied most thoroughly, and designated T993, 41 females of $X'X^t$ constitution yielded 12% sterile exceptional sons, the remaining 88% of the progeny being regular females. Similarly in tests on 32 XX^t females, 5% of the offspring were exceptional daughters and 95% were regular sons.

Presented in the diagrams in Figs. 3 and 4 are some of the egg types which females of $X'X^t$ and XX^t constitution respectively would be expected to mature. Not all of the theoretically possible kinds of disjunction are considered, only those which are believed to yield viable progeny.

According to this scheme, alternate 2:2 disjunction gives rise to offspring of the expected sex, whereas 3:1 disjunction of the type

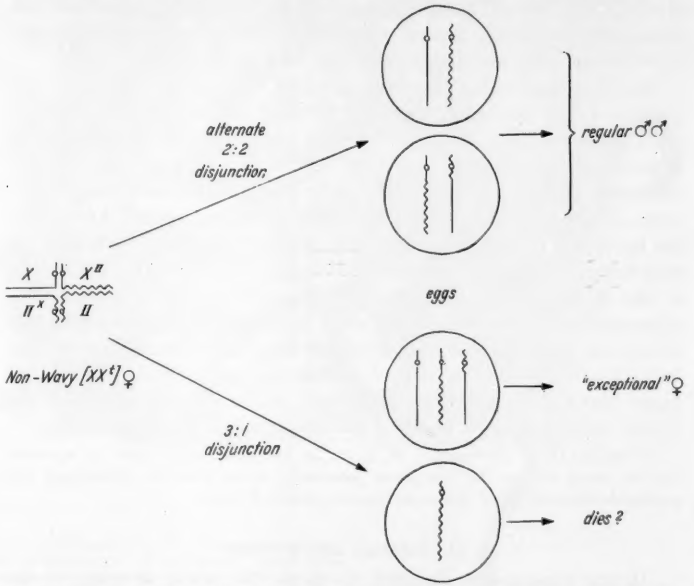


Fig. 4. Diagram of the egg types formed by male-producers heterozygous for PT1. The normal X carries the wild-type allele of *Wavy*; *swollen* is present on the translocated X, and *swollen* may or may not be present on the normal X

illustrated produces the exceptional sexes. The exceptional males are believed to arise from those eggs which receive no sex chromosome (Fig. 3), and the exceptional females from eggs which receive two (Fig. 4). Implicit in this scheme is the view that the influence of the X' chromosome on sex of progeny is an indirect one: the presence of X' in the genetic make-up of a female results in the elimination during cleavage of one paternal X instead of two from the embryonic somatic nuclei of all her eggs. If, following this elimination, two sex chromosomes remain, the embryo becomes a female; if only one remains, the embryo becomes an exceptional male. Thus in the case of eggs which receive no

sex chromosomes during oogenesis, only the two X's from the sperm are delivered to the zygote; following chromosome elimination, a single sex chromosome remains in the somatic nuclei and consequently the embryo develops into a male.

Applying the same scheme to male families, the absence of an X′ chromosome in the genetic make-up of a mother results in the elimination of both paternal X's from the embryonic soma, leading to the production of sons. If, however, two sex chromosomes (Fig. 4) instead of one are distributed to the egg during oogenesis, two sex chromosomes remain in the soma after elimination and the embryo becomes a female.

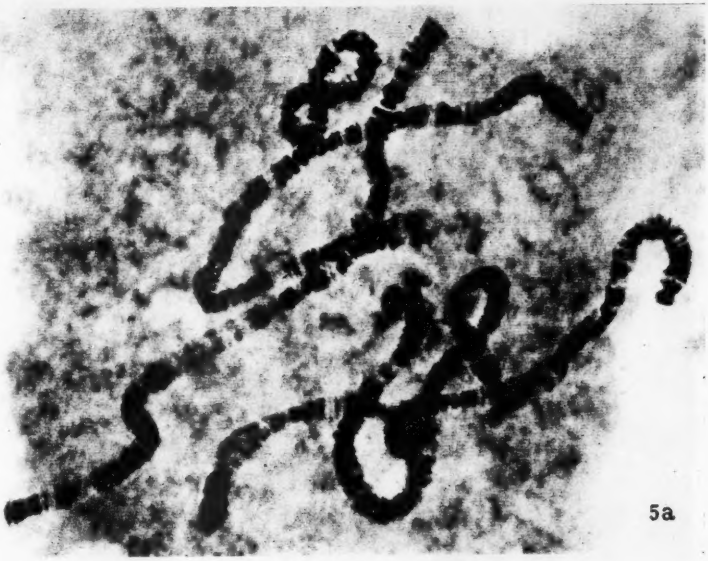
The question should be raised as to whether some of the non-disjunctional eggs shown in Figs. 3 and 4 might develop into the expected sexes, namely, those eggs laid by XX^{l} females which receive no sex chromosome and those laid by $X'X^{l}$ females which receive two. Would the former develop into males distinguishable from their brothers only by virtue of the patroclinous nature of their sex-linked genes? And would the latter develop into females distinguishable from their sisters only by their failure to inherit a paternal X? Recalling the nature of the influence of the X′ chromosome on the sex of progeny, i.e. on chromosome elimination from the embryonic soma, we find that the former group of zygotes would possess no sex chromosome after elimination and the latter would possess three. Hence, neither type of egg would be expected to be viable. Therefore the percentage of the exceptional sex types should provide a valid index of the frequency of non-disjunction.

I wish to thank Professor C. W. METZ for his continued interest in my work and for many favors. He has given generously of his time for stimulating and profitable discussions of the experiments presented here.

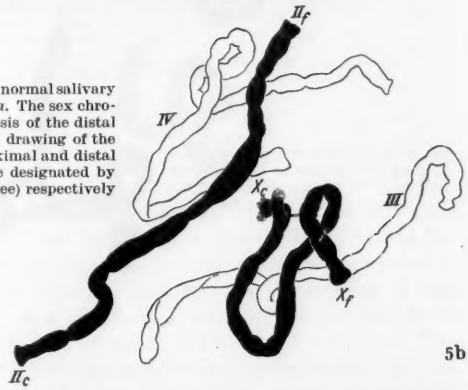
II. Materials and Methods

In the experiments designed to clarify the mode of origin of the exceptional sexes, an X-translocation was sought which would be viable in both the homozygous and heterozygous condition and which could be combined with other available chromosomal aberrations to produce varying amounts of non-disjunction. These studies were handicapped by a dearth of genetic markers; only two sex-linked mutants and one mutant per autosome were available. The translocation selected for study was one between X and chromosome II, designated PT1 (for nomenclature and cytological maps of the chromosomes of *S. coprophila*, see CROUSE 1943). Both X and II have sub-terminal centromeres. The exact position of centromeres on the salivary gland chromosomes of *Sciara* cannot be determined by microscopic study: there is no chromocenter in these nuclei, and the centric regions are not characterized by any visible differential quality. Knowing which salivary element represents the only V-shaped chromosome of the complement ($n=4$), it has

been possible, by studying a group of reciprocal translocations (CROUSE 1943) to deduce which end is proximal on the three remaining chromosomes. Thus for chromosomes X and II respectively, the free ends are



5a



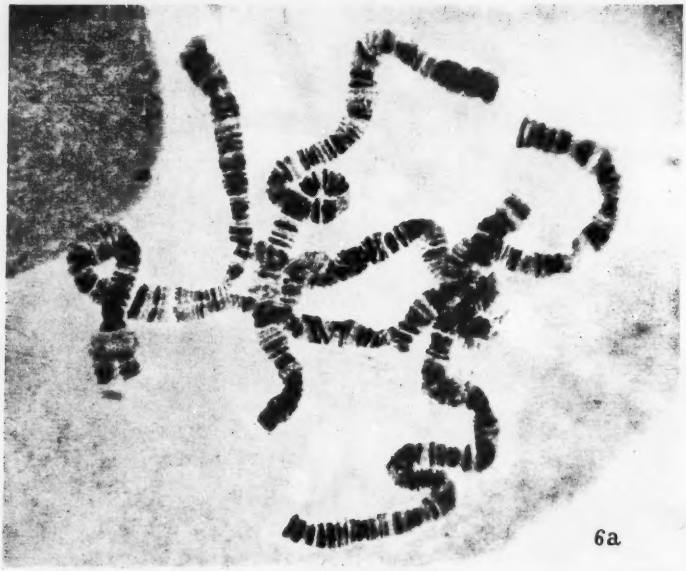
5b

Fig. 5. (a) Photomicrograph of the normal salivary gland complement of *S. coprophila*. The sex chromosome is tied in a loop by synapsis of the distal and proximal repeats. (b) Outline drawing of the chromosomes in Fig. 5a. The proximal and distal ends of chromosomes X and II are designated by the letters *c* (centromere) and *f* (free) respectively

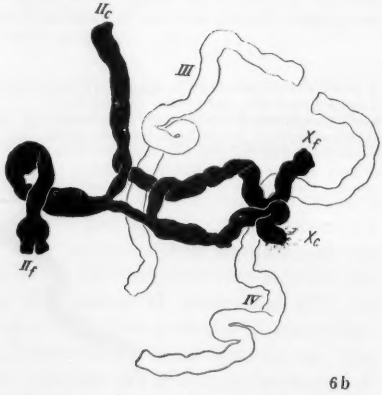
located in salivary map regions 1A and 13A, and the proximal ends in regions 12C and 22C.

Translocation PT1 was obtained in the following manner: adult *swollen* males were x-rayed at 4000 r and bred to X'X females, hetero-

zygous for *Wavy*. *Wavy*, it will be recalled, is a dominant located on an X′ chromosome, and *swollen*, a recessive located on an X. The F_1 *Wavy* daughters (heterozygous for *Wavy* and for *swollen*) were in turn



6a



6b

Fig. 6. (a) Photomicrograph of translocation PT1 as seen in heterozygous condition. (b) Outline drawing of the chromosomes in Fig. 6a

bred singly to wild-type males and their progeny examined for exceptional sons. One culture yielded 6 exceptional wild-type males in addition to the expected *Wavy* (23) and wild-type (22) dughters. The presence of males in a female culture and the fact that the males were patro-

clinous for their sex-linked genes, suggested that the mother of this culture was heterozygous for an X-translocation. Subsequent cytological study of the salivary gland chromosomes revealed translocation PT1.

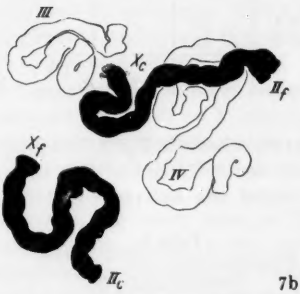


Fig. 7. (a) Photomicrograph of translocation PT1 as seen in homozygous condition. The proximal and distal repeats of the sex chromosome are not synapsed, but a lateral connection is visible between the proximal and the interstitial repeat. (b) Outline drawing of the chromosomes in Fig. 7a

The salivary gland chromosomes of normal *S. coprophila* are shown in Fig. 5a and those of the heterozygous translocation in Fig. 6a. Outline drawings of the chromosomes in these nuclei are presented in Figs. 5b and 6b. It will be noted that the sex chromosome of this species is normally tied in a loop configuration by lateral synapsis of two repeat regions which lie near either end of the chromosome. The repeats are composed of three bands, a doublet and a singlet; the one at the proximal end is located in map region 12B and the one near the distal end in map region 2C. Actually there is a third, or interstitial, repeat on this chromosome which often does not reveal itself. It is located in map region 10B and can be seen in Figs. 7 and 8 by virtue

of its synapsis with the proximal repeat. In translocation PTl, the point of breakage on chromosome X is in map region 8B and that on chromosome II in region 18B. The two rod-like chromosomes produced by the reciprocal translocation were found to be indistinguishable at metaphase from the original X and II. The homozygous translocation is shown in Fig. 7.

III. Results

Various genetic studies have been conducted on PTl, all with the view to varying the chromosome configuration in the nucleus and thereby altering the frequency of non-disjunction during oogenesis. The results of these studies are presented below.

1. Exceptional Males Derived from X'X^t Mothers

X'X^t females, heterozygous for *Wavy* (X') and for *swollen* (X^t), were bred singly to wild-type males. Two kinds of progeny were obtained: *Wavy* and wild-type daughters and exceptional wild-type sons. Because the translocation was kept in stock for more than two years, this kind of cross was repeated many times and hundreds of exceptional sons obtained. In every instance they were patroclinous for their sex-linked genes, thereby fulfilling the expectancy according to the scheme in Fig. 3. The progeny from twenty-five X'X^t mothers are recorded in Table 1 to show the average percentage (9.1%) of exceptional males and the range (2.5% to 17.3%) from culture to culture.

Table 1. *Progenies of X'(W)X^t (sw)* ♀♀ *Bred to* + ♂♂

Culture	*Wavy* and + ♀♀	exc. + ♂♂	% exc. ♂♂
A 255-4	39	1	2.5%
A 260-6	43	9	17.3%
Totals for 25 cultures	1211	121	$\frac{121}{1332} = 9.1\%$

Table 2
Progenies of X'(+)X^t (sw) ♀♀
Bred to Fused ♂♂

Culture	*Fused* ♀♀	exc. *Fused* ♂♂
A 87-2	10	1
-3	47	2
-4	32	3
-5	41	1

Table 3. *Progenies of X'(W)X^t (sw) Fused (11)* ♀♀ *Bred to Normal swollen* ♂♂

Culture	♀♀		exc. *F sw* ♂♂
	WF	*sw*	
A 276-1	27	22	2
-2	15	16	1
-3	20	23	1
A 298-1	16	15	3
-2	20	21	5

Using the data recorded in Tables 1, 2, and 3, a graph was made of the percentage of exceptional males per culture. This graph was found to approximate a normal curve. Hence, the fluctuation in percentage

of exceptional males from culture to culture is deemed not to be significant.

According to the scheme in Fig. 3, the exceptional males should receive a normal chromosome II from both parents. Use was made of the autosomal dominant *Fused*, located on chromosome II, to show that this is the case. Table 2 records data obtained by breeding $X'X^t$ females to *Fused* males. As expected, all the offspring, including the exceptional males, showed the *Fused* wing character. In Table 3 are recorded data obtained by breeding $X'X^t$ females, heterozygous for *Wavy* (X'), *swollen* (X^t), and for *Fused* (II) to normal (i.e., no translocation present) *swollen* males. The chromosomal configuration of these females is like that shown in Fig. 3, the *Fused* marker being present on the normal chromosome II. Two kinds of daughters are expected from such a cross, *Wavy-Fused* and *swollen*; and the exceptional males are expected to be *swollen Fused*. The data obtained are recorded in Table 3 and it will be noted that they fit this expectancy.

2. Exceptional Females Derived from XX^t Mothers

According to the scheme in Fig. 4, the exceptional females derived from XX^t mothers arise from those non-disjunctional eggs which receive both the normal and the translocated sex chromosomes. Hence if XX females homozygous for *swollen* were bred to wild-type males, one would expect their exceptional daughters to be *swollen*, provided both of the paternal X's are eliminated from the embryonic somatic nuclei as is ordinarily the case for eggs laid by females which lack an X' chromosome in their genetic make-up. The progeny from twenty-five matings of this kind are recorded in Table 4. It will be noted that both *swollen* and wild-type exceptional daughters occurred and that the former were approximately three times as frequent as the latter (ratio of 26:10). The occurrence of exceptional wild-type daughters in the progeny indicates that the pattern of chromosome elimination from the embryonic soma of these eggs was altered and that instead of eliminating both paternal X's, at least one paternal X was retained. That errors in elimination might arise in such non-disjunctional eggs is not difficult to imagine; for, following fertilization the zygote nucleus contains four sex chromosomes instead of the usual three. Because these eggs occurred at such low frequencies, it was not feasible to make a cytological study of chromosome elimination, but examination of the salivary gland complement of large numbers of exceptional females showed that they were disomic, not trisomic, for the sex chromosome. Thus the predominance of exceptional daughters of the swollen phenotype (Table 4) supports the scheme diagrammed in Fig. 4; and the occurrence of wild-type excep-

tional females suggests errors in elimination from the somatic nuclei during cleavage.

Because the elimination of the sex chromosome from the germ line occurs much later in development and is the same in both sexes, and because the germ line of *Sciara* can apparently tolerate extra chromatin in the nucleus in the form of the "limited" chromosomes, the question

Table 4. *Progenies of X(sw)X^{l} (sw) ♀♀ Bred to + ♂♂*

Culture	*sw* ♂♂	exc. ♀♀		% exc. ♀♀
		+	*sw*	
A 135-1	45	0	0	0.0%
A 126-10	33	3	4	17.5%
Totals for 25 cultures	691	10	26	$\frac{36}{727} = 0.049 = 4.9\%$

arises as to the germ line constitution of the exceptional daughters recorded in Table 4. Following the elimination of one paternal X from the germ cells of these embryos, three sex chromosomes should remain: the two maternal ones, each carrying *swollen*, and a paternal X carrying the wild-type allele. Twenty-three of the exceptional *swollen* females recorded in Table 4 were bred to their *swollen* siblings. Only seven produced offspring, but among the progeny of all of them wild-type flies were obtained (see Table 5). Six of the seven yielded exceptional daughters as would be expected, if their germ line were trisomic for the sex chromosome. One of the exceptional *swollen* females (Culture A162-1 in Table 5) failed to produce exceptional daughters; presumably only two sex chromosomes had been retained in her germ line, a maternal and a paternal X.

Table 5. *Progenies of Exceptional sw ♀♀ (recorded in Table 4) Bred to sw ♂ Sibs*

Culture	♀♀		♂♂	
	+	*sw*	+	*sw*
A 148-1	1	0	0	1
A 149-1	1	0	1	2
-2	1	1	1	5
A 153-1	4	0	3	1
A 157-1	2	2	8	3
-2	9	3	1	5
A 162-1	0	0	37	46

According to the scheme presented in Figs. 3 and 4, the exceptional males and females arise from the same type of non-disjunction. Nevertheless the frequency of exceptional females is only 4.9% (Table 4) as compared to 9.1% exceptional males (Table 1). There are two factors which may contribute to this inequality. In the first place, the X′ chromosomes in the stocks of *S. coprophila* which were used for these studies, carried a long paracentric inversion (unpublished studies by the author). This inversion shown in Figs. 8a and b extends practically the entire length of the chromosome. To what extent it impedes meiotic pairing is not known, but in the salivary gland nuclei the heterozygous

8a

8b

Fig. 8. (a) Photomicrograph of the salivary gland chromosomes of an X′X female showing the long paracentric inversion present on the X′ chromosome in the stocks of *S. coprophila* used for these studies. (b) Outline drawing of the chromosomes in Fig. 8a

figures are mostly unanalyzable. Assuming that the inversion does interfere with synapsis during oogenesis, the frequency of non-disjunction in X^tX^t females should exceed that in XX^t females, and hence the percentage of exceptional sons should be greater. Thus the higher frequency of exceptional males lends support to the view that the exceptional sexes originate from 3:1 disjunction. The second factor which may contribute to the inequality is the greater likelihood of recovering eggs which have no sex chromosome as compared to the recovery of eggs which have two.

3. The Progeny Derived from X^tX^t Mothers

The homozygous translocation is shown in Figs. 7a and b, and, as one would expect, the homologous portions of X and of II are tightly paired. With this chromosomal configuration there should be no obstruction to normal synapsis and disjunction of chromosomes during oogenesis, and hence one would not expect to find exceptional females in the families of X^tX^t mothers.

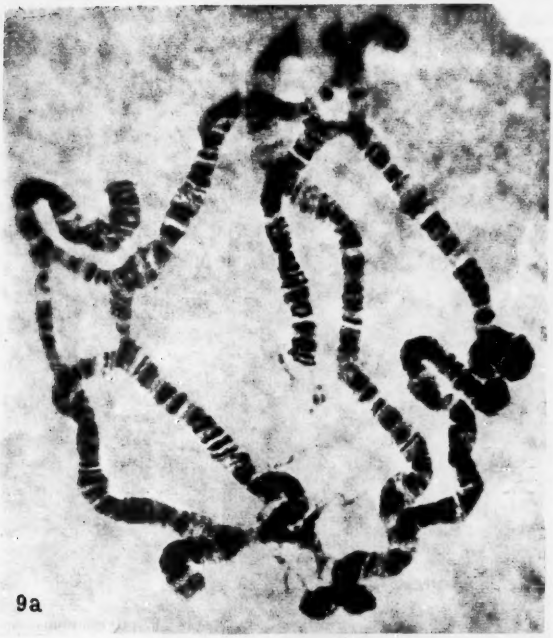
Table 6
Progenies of X^tX^t ♀♀ Bred to Wild-type ♂♂

Culture	*sw* ♂♂	exc. ♀♀
A 54-6	20	0
-7	27	0
A 60-4	11	0
-5	17	0
A 70-3	67	0
A 73-2	63	0
A 79-4	22	0
-5	26	0
A 80-1	84	0
-2	72	0
-3	52	0
-4	66	0
-5	50	0
-6	42	0
-7	48	0
-8	80	0
-9	64	0
A 82-3	52	0
-4	49	0
A 91-3	22	0
	934	0

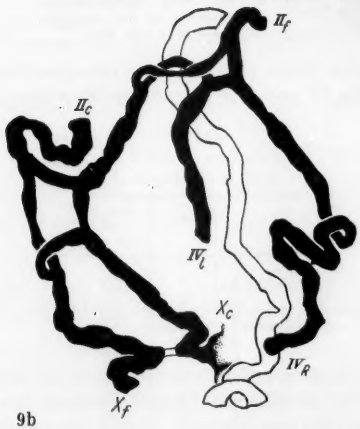
Forty-seven *swollen* females, homozygous for PTl, were bred to wildtype males. Of these, twenty yielded offspring. Their progeny are recorded in Table 6. There was a total of 934 *swollen* sons and no exceptional daughters.

4. The Progeny Derived from Mothers Heterozygous for "Stop" and for PTl

In the genetic studies on PTl, use was made of "Stop", a reciprocal translocation between autosomes II and IV which acts as a recessive lethal and which in heterozygous condition produces a visible wing character. It is shown in Figs. 10a and b. The break on chromosome II is in map region 14C and that on IV in map region 39B (CROUSE 1943). By combining *Stop* with PTl, a chromosomal configuration was produced similar to that shown in the photomicrograph and drawing in Figs. 9a and b. Females of this genotype proved to be highly inviable and partially sterile. A few yielded progeny (Tables 7 and 8), but the data are too meagre to allow any conclusion regarding the effect of *Stop* on the frequency of the exceptional sex types. There are, however, two points of interest with reference to these data, namely, that the excep-



9a



9b

Fig. 9. (a) Photomicrograph of the salivary gland chromosomes of a female heterozygous for PT1 and for the *Stop* translocation. (b) Outline drawing of the chromosomes in Fig. 9a

tional males are all *Stop* and that none of the exceptional females in *Stop*. This is exactly what would be expected following non-disjunctios

of the sex chromosomes. For, if we examine the complement of the *Stop* PTl mothers (Fig. 9) and consider the viable egg types formed by

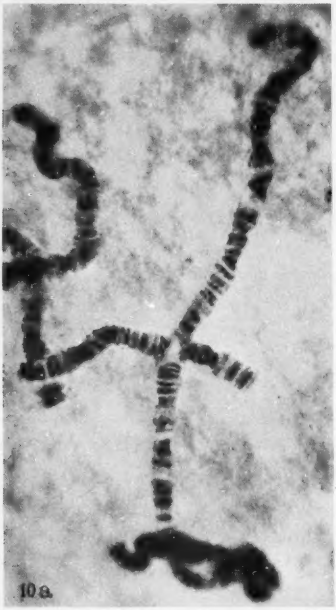


Fig. 10. (a) Photomicrograph of the *Stop* translocation. (b) Drawing of the chromosomes in Fig. 10a

non-disjunction of sex chromosomes, those eggs receiving chromosomes X^{II}, X, and II^{X} should also receive IV and give rise to non-Stop exceptional daughters; whereas the complementary type of egg, receiving chromosomes II^{IV} and IV^{II}, should develop into exceptional *Stop* sons.

Table 7
Progenies of X'X^{t}S ♀♀
Bred to Wild-type ♂♂

Culture	*S* ♀♀ and + ♀♀	exc. *S* ♂♂
A 315-3	32	1
-5	20	3
A 319-2	29	4

Table 8
Progenies of XX^{t}S ♀♀
Bred to Wild-type ♂♂

Culture	exc. + ♀♀	S ♂♂ and *sw* ♂♂
A 311-4	1	20
-6	1	12
A 315-4	0	23
-6	1	36
-9	0	15
A 318-3	1	31
A 319-1	1	28
-4	0	23

5. The Progeny Derived from Stop Females Bred to PTl Males

Quite unexpectedly during the cross-breeding of *Stop* and PTl, a few *Stop* sons were obtained from male-producing *Stop* females bred to PTl males (Table 9). It was stated above that when X-translocations were taken through the male germ line, no sons were recovered, presumably because of the unbalanced chromosome complement in the somatic nuclei following elimination (see text, p. 149 and Fig. 2). The same was found to be the case when PTl males were bred to normal male-producers. That the mating of XX *Stop* females to PTl males should yield *Stop* sons was thus wholly unexpected. These sons were the result of 3:1 disjunction during oogenesis, as will now be demonstrated.

Table 9
Progenies of XXS ♀♀
Bred to swollen PTl ♂♂

Culture	exc. ♀♀	*S* ♂♂
A 282-5	0	7
A 363-1	0	6
-2	0	5
-3	0	4
-4	0	3
-5	0	7
-6	0	5
-7	0	5
-8	0	6
-9	0	7

Diagrammed in Fig. 11 are the chromosomes involved in this cross. Heretofore it has not been necessary to consider the chromosome complement of the sperm matured by PTl males. It was stated above

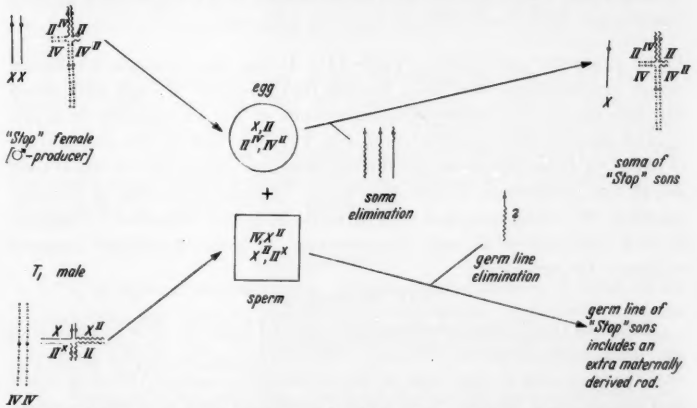


Fig. 11. Diagram of the chromosomes inherited and eliminated when male-producers heterozygous for *Stop* are bred to PTl males

that on the basis of previous studies, the mode of transmission of X-translocations through the male could be represented diagramatically as shown in Fig. 2. Spermatogenesis has been studied cytologically in PTl males (unpublished) and found to follow this same pattern. In the diagram in Fig. 11 the sperm complement is enclosed in a square and

that of the egg in a circle. The exact nature of the complements was deduced from the following lines of evidence. The salivary gland nuclei of the sons showed a normal X and the *Stop* translocation. That the normal chromosome IV in the somatic complement of the *Stop* sons was not of maternal origin is clear from the data in Table 10. Use was made of the dominant marker *Dash* located on IV. Thus, when females of the genotype XX *SD* were bred to PTl males, none of the *Stop* sons showed the *Dash* character. Use was also made of the dominant marker *Fused* located on chromosome II to show that the normal II in the somatic complement of the *Stop* sons was not of paternal origin. When XX *SF* females were bred to PTl males, all of the *Stop* sons showed the

Table 10. *Progenies of XXSD ♀♀ Bred to swollen PTl ♂♂*

Culture	exc. ♀♀	*S* ♂♂
A 381-1	0	4
-2	0	7
-4	0	7
-5	0	7
-6	0	2
-7	0	5

Table 11. *Progenies of XXSF ♀♀ Bred to swollen PTl ♂♂*

Culture	exc. ♀♀	*SF* ♂♂
A 344-1	0	2
-2	0	1
-4	0	1
-5	0	2

Fused character (see data in Table 11). It was thus possible to deduce which chromosomes had been transmitted through the egg and which through the sperm; clearly the occurrence of 3:1 disjunction in a few eggs of *Stop* mothers (indicated in Fig. 11), followed by the elimination of three paternal chromosomes during cleavage instead of the usual two, led to the production of the *Stop* sons. Although this case of non-disjunction of autosomes does not constitute direct evidence in support of 3:1 disjunction of sex chromosomes, it does constitute indirect evidence by analogy.

IV. Discussion

Whereas the X-translocations have proved to be of value in analyzing the mechanism of control of the sex chromosome at the second spermatocyte division and in the cells of the developing embryo (CROUSE 1943, and unpublished studies), they have contributed little to a real clarification of the genetic device which governs sex of progeny. That the exceptional sex types associated with the translocations are the result of non-disjunction of sex chromosomes during oogenesis and not the result of a agenetic change induced by rearrangement of the X, seems clear from the data presented above. The translocation studies support the view (for a thorough discussion of theories of sex determination, in *Sciara*, see METZ 1938) that the influence of the X′ chromosome in

monogenic *S. coprophila* is not one on sex of progeny *per se*, but rather a determinant governing the elimination from the embryonic soma of one paternal X as opposed to two. The chromosome group which remains in the soma determines the sex of the individual fly: if two sex chromosomes remain, the differentiation of the gonad into an ovary proceeds normally even when the germ cell nuclei contain three X's (data in Table 5, and REYNOLDS 1938); and if only one sex chromosome (maternal) remains, the gonad differentiates into a normal testis even when the germ cell nuclei contain an extra autosome (see Fig. 11). That errors in the kind and number of chromosomes eliminated from the embryonic soma do indeed occur, seems evident from the data presented above.

At present we do not have a satisfactory, comprehensive theory of sex determination in *Sciara*. It awaits our understanding of the genetic nature of X′ and how X′ maintains itself intact. With this knowledge, perhaps we will be in a position to understand the evolutionary steps involved in the origin of the digenic condition characteristic of some species of *Sciara* in which the sex rations may be highly variable.

The induction and consequences of a variety of chromosomal conditions in *S. coprophila* will be considered fully in subsequent publications, also the effect of the paternal X on testis development in the exceptional males.

A case of non-disjunction of sex chromosomes similar to the one described above was observed by DOBZHANSKY (1932) in *Drosophila* males heterozygous for a series of Y-II translocations. In this case, patroclinous (patroclinous for genes located on chromosome II) sterile XO males were produced by those sperm which failed to receive any sex chromosome as result of 3:1 disjunction.

Summary

1. A reciprocal translocation between chromosomes X and II in *S. coprophila* was used to elucidate the occurrence of exceptional sons and daughters among the progeny of females heterozygous for any one of several X-translocations.

2. The studies support the view that the exceptions result from non-disjunction of sex chromosomes during oogenesis and not from genetic change induced by rearrangement on the X.

3. The percentage of exceptions was found to increase when chromosomal rearrangements were used to decrease synapsis of the sex chromosomes. Conversely, when the barrier to sex chromosome pairing was removed, by making the translocation homozygous, the exceptions did not occur.

4. The studies support the view that (1) the X′ influence on sex of progeny is one on sex chromosome elimination from the embryonic soma, and (2) sex of the individual fly is determined by the chromosome complement which remains in the soma after elimination.

5. Provided there are two sex chromosomes in the somatic complement, differentiation of the ovary is normal even when an extra sex chromosome is present in the germ line. Likewise, the testis differentiates normally provided the somatic complement is XO (maternal X) even when an extra autosome is present in the germ cells.

6. When an additional sex chromosome is transmitted to the zygote (i.e., 4 sex chromosomes present), errors may occur in the kind or the number of chromosomes eliminated from the embryonic soma. No errors in elimination of chromosomes from the germ line have been detected.

References Cited

BERRY, R. O.: Observations on chromosome elimination in the germ cells of *Sciara ocellaris*. J. Morph. **68**, 547—583 (1941).

CROUSE, H. V.: Translocations in *Sciara*; their bearing on chromosome behavior and sex determination. Univ. Missouri Res. Bull. **379**, 1—75 (1943).

DOBZHANSKY, TH.: Studies on chromosome conjugation. I. Translocations involving the second and the Y-chromosomes of *Drosophila melanogaster*. Z. indukt. Abstamm.- u. Vererb.-Lehre **60**, 236—288 (1932).

METZ, C. W.: Chromosome behavior, inheritance, and sex determination in *Sciara*. Amer. Naturalist **72**, 485—520 (1938).

REYNOLDS, J. P.: Sex determination in a bisexual strain of *Sciara coprophila* LINTNER. Genetics **23**, 203—220 (1938).

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RATES OF MEIOSIS, SPINDLE IRREGULARITIES AND MICROSPOROCYTE DIVISION IN BROMUS TRINII × B. CARINATUS

By

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With 54 Figures in the Text

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I. Introduction

The combination of two genomes in an interspecific hybrid has frequently resulted in aberrant meiotic divisions. Probably all the types of irregularities that have been produced experimentally have been expressed spontaneously in interspecific hybrids.

Meiotic aberrations have been particularly abundant in interspecific hybrids of *Bromus*. Those concerned with chromosome movements and with the organization of the spindle were previously reported in the hybrid *B. rubens* × *carinatus* (WALTERS 1958). A more recent hybrid, *B. trinii* × *carinatus*, exhibits a complex of aberrations relating to chromosome distribution, spindle organization, microsporocyte division, and rates of meiosis. These aberrations vary in expression among individual anthers.

The present investigation has several objectives: First, the interrelationships of aberrant chromosome movement, spindle organization, and microsporocyte division can be investigated and these related to the changed meiotic rates. Second, observations of abnormal meiotic divisions expose extremes of behavior that the cell is capable of manifesting. A determination of possible limits of deviation from the normal patterns of structure and function provides some information concerning characteristics of normal meiotic divisions. And finally, since the fundamental processes by which chromosome reapportionment and cell division take place are still not completely known, an important objective remains the careful description of normal and aberrant details of the meiotic divisions.

II. Materials and Methods

The hybrid used in this study involved the species *Bromus trinii* ($2n = 42$) of the section *Neobromus*, and *B. carinatus* ($2n = 56$) of the section *Ceratochloa* (for detailed relationships see WALTERS 1957).

B. trinii was collected near Esquel, Chubut, Argentina. *B. carinatus* was obtained in Santa Barbara. The hybrid *B. trinii* × *carinatus* (2n = 49) was grown in the field in Santa Barbara.

Fixations for cytological study were taken from the first flowering spike of each plant. Fixations were made in a mixture of 3 parts absolute alcohol : 1 part glacial acetic acid and stored in 70% alcohol under refrigeration. Meiosis was studied in iron-acetocarmine smears of microsporocytes. The room was humidified with steam before these slides were made. In a humid atmosphere the aceto-carmine evaporates very slowly and plenty of time is available for careful macera-tion of all portions of the anther and removal of anther wall debris. When it was desirable to count all the microsporocytes in an anther, slides were made as follows: One-third of an anther was gently macerated in a very small drop of acetocarmine; pieces of anther wall were moved over to one or two adjacent droplets and the maceration repeated (this prevents crushing of microsporocytes already liberated); anther wall debris was removed by carefully straining it away from the liquid with a needle; the cover slip was then placed. If the final drop of acetocarmine is small enough that it forms a thin film extending only to the edge of the coverslip, the coverslip need not be pressed, thus avoiding the possibility of loss of any microsporocytes at the edges. Gentle heating is enough to flatten the micro-sporocytes and prevent their too rapid darkening. The efficacy of this method is shown by examining the discarded anther debris in a separate drop of aceto-carmine; very few microsporocytes are found. For general studies of meiosis more heat was applied and microsporocytes were flattened by some pressure on the cover slip.

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III. Observations

Distribution of Meiotic Stages

Anthers were studied in which the ratios of microsporocytes in meiosis-I and meiosis-II ranged from 88:1 to 1:26. The different stages of meiosis occurred in various proportions in individual anthers (see Table 1). These hybrid anthers have a wider range of meiotic stages than is usual in *Bromus*. In very young anthers the range is narrow, perhaps from zygotene to a few metaphase-I cells or zygotene to a few prophase-II cells. In older anthers the range increases, e.g., from leptotene to numerous metaphase-II cells. In still older anthers it may be so extensive as leptotene to tetrads or zygotene to microspores, with the majority of cells in the later meiotic stages.

From the beginning of meiosis the anther is progressively older from base to apex. The lower half of a very young anther (zygotene to pro-phase-II) contains mostly cells in early prophase-I; the upper half (pachytene to metaphase-II) contains mostly cells in mid-late pro-phase-I. The lower third of an older anther is pachytene to metaphase-II,

the middle third diplotene to late anaphase-II, the upper third diakinesis to tetrads. The proportions of cells in the later meiotic stages increase markedly from the lower to the upper regions of the anther; in one anther the bottom third contains 0.12 anaphase-II to tetrad cells, the middle third 0.28, the top 0.52. A clump of microsporocytes may exhibit the whole range of meiotic stages characteristic of the anther. Thus there is apparently no further localization of certain stages within the anther.

Anthers from the same floret are usually similar in meiotic development, e.g., Table 1, anthers W, AA, DD; N, Q, R. Sometimes, however, striking differences are observed; e.g., C is much younger than H and CC is much younger than II.

Great variation is found in the proportions of different meiotic stages in anthers containing the same range of stages. Anthers with the same ratios of meiosis-I:II cells frequently exhibit similar proportions of cells in the various meiotic stages; e.g., compare CC, DD; Q, R, AA, BB; FF, HH. However, great differences may occur in anthers with the same overall meiosis-I:II ratio; e.g., compare L, M; P, T; Z, AA.

The generally very low proportions of anaphase-I microsporocytes indicate that this is usually a very short stage. However, it is prolonged in anther T and particularly in I. Late telophase-I and interphase-I were present in a few anthers, but usually early telophase-I (and in one anther late anaphase-I) is transformed directly into prophase-II. The proportion of telophase-I and prophase-II cells varied from 0.03 to 0.30 in anthers with the same (2:1) overall meiotic ratio. The distribution of stages in anther O is unusual in that there is a high proportion of prophase-I and anaphase-telophase-II cells, and very low proportions of the remaining meiotic stages.

Chromosome Distribution

At diakinesis the chromosomes are well spread. At pro-metaphase they form a compact round ball in the center of the cell; it is not possible to separate and flatten the chromosomes at this stage. This pro-metaphase chromosome clumping, termed the "mitotic contraction stage" by Bajer and Molè-Bajer (1956) was also observed in meiosis in *Bromus rubens* × *carinatus* (Walters 1958).

At metaphase the bivalents are oriented on the equatorial plate and many of the univalents may be near the poles (see also Walters 1958). The average bivalent frequency in four anthers was 11.9—13.6 bivalents per cell; the range was 6—18. The mode was 13—15 bivalents per cell and therefore about 20 univalents are most often found in each cell.

The frequencies of lagging chromosomes were compared at late anaphase-I, early telophase-I and prophase-II (Table 2). For this

Table 1. *Proportions of meiotic stages and of multinucleate microsporocytes in individual anthers (arranged in order of ratio, meiosis I : meiosis II)*

Plant number	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
Floret number	2—4	2—5	2—1	2—1	2—3	2—3	2—3	2—1	2—3	2—4	2—1	2—3	2—3	2—3	2—3	2—3	2—3	2—3
Anther number	1	2	2	3	9	6	1	2	1	2	7	5	7	8	2	5	8	8
	a	a	c	a	a	a	a	b	b	a	b	c	a	b	b	b	a	c
Meiotic stages																		
Pro-I	0.94	0.83	0.45	0.41	0.84	0.81	0.30	0.31	0.07	0.67	0.51	0.61	0.19	0.35	0.62	0.41	0.31	0.37
M-I	0.03	0.08	0.43	0.43	0.05	0.06	0.42	0.45	0.49	0.05	0.17	0.04	0.32	0.16	0.01	0.05	0.13	0.13
A-I	0.01	0.02	0.06	0.05	0.02	0.03	0.10	0.07	0.21	0.03	0.06	0.03	0.10	0.08	0.01	0.03	0.08	0.09
T-I + Pro-II	0.02	0.05	0.02	0.09	0.05	0.06	0.13	0.10	0.21	0.07	0.13	0.09	0.30	0.13	0.03	0.10	0.14	0.10
M-II	0.01	0.01	0.01	0.01	0.02	0.02	0.03	0.03	0.02	0.06	0.06	0.08	0.07	0.15	0.04	0.09	0.17	0.13
A + T-II	0.00	0.00	0.03	0.01	0.01	0.01	0.01	0.05	0.00	0.09	0.06	0.11	0.02	0.08	0.24	0.15	0.09	0.12
Tetrads	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.05	0.00	0.04	0.04	0.16	0.08	0.07
Total no. of cells	1983	1734	2221	1738	2319	2348	2597	2459	2311	2187	2592	2320	2067	2406	2611	2135	2392	2486
Ratio, meiosis I:II[1]	59:1	23:1	17:1	15:1	13:1	11:1	7:1	6:1	5:1	3:1	3:1	2:1	2:1	2:1	2:1	1:1	1:1	1:1
Multinucleate microsporocytes																		
Pro-II	0.00	0.00	0.00	0.00	0.03	0.02	0.02	0.03	0.00	0.07	0.01	0.06	0.01	0.01	0.17	0.11	0.04	0.03
T-II	—	—	0.04	—	—	—	—	0.05	—	0.05	0.00	0.22	—	0.05	0.08	0.19	0.05	0.04
No. of microsporocytes																		
Pro-II	14	42	38	50	74	92	135	113	185	99	213	163	499	195	65	178	242	183
T-II	—	—	24	—	—	—	—	43	—	83	47	109	—	44	300	130	59	107

Table 1 (Continued)

Plant number Floret number Anther number	S 2—1 7 a	T 1—1 6 a	U 1—1 11 a	V 2—1 1 a	W 2—1 6 c	X 2—3 2 c	Y 1—2 1 a	Z 2—1 4 a	AA 2—1 6 b	BB 1—1 8 a	CC 1—1 7 a	DD 2—1 6 a	EE 2—2 2 a	FF 1—1 4 a	GG 2—2 1 a	HH 1—1 9 a	II 1—1 7 c
Meiotic stages																	
Pro-I	0.19	0.04	0.21	0.20	0.28	0.23	0.32	0.10	0.19	0.08	0.13	0.16	0.09	0.04	0.08	0.02	0.02
M-I	0.12	0.26	0.15	0.04	0.01	0.03	0.02	0.08	0.02	0.12	0.05	0.01	0.02	0.02	0.01	0.01	0.01
A-I	0.07	0.16	0.05	0.02	0.01	0.03	0.02	0.04	0.02	0.05	0.02	0.01	0.01	0.01	0.01	0.01	0.00
T-I + Pro-II	0.25	0.26	0.28	0.11	0.04	0.08	0.12	0.18	0.05	0.09	0.08	0.03	0.01	0.05	0.03	0.03	0.04
M-II	0.13	0.22	0.11	0.12	0.05	0.09	0.05	0.13	0.10	0.12	0.02	0.06	0.02	0.02	0.05	0.04	0.03
A + T-II	0.19	0.05	0.07	0.20	0.32	0.35	0.26	0.34	0.28	0.24	0.25	0.32	0.29	0.13	0.22	0.18	0.25
Tetrads	0.05	0.02	0.12	0.32	0.29	0.19	0.20	0.13	0.33	0.30	0.46	0.40	0.56	0.72	0.60	0.71	0.64
Total no. of cells	2367	2363	2207	1885	2168	2407	2831	1955	1988	2179	1893	1927	2139	1995	1980	2422	2086
Ratio, meiosis I:II[1]	1:1	1:1	1:1	1:2	1:2	1:2	1:2	1:3	1:3	1:3	1:4	1:4	1:8	1:13	1:19	1:22	1:26
Multinucleate microsporocytes																	
Pro-II	0.02	0.00	0.03	0.10	0.15	0.11	0.16	0.01	0.22	0.00	0.09	0.14	0.36	0.14	0.17 to 0.24	0.02	0.16
T-II	0.02	0.00	0.05	0.16	0.11	0.09	0.12	0.02	0.12	0.02	0.21	0.10	0.09	0.40	0.20	0.06	0.32
No. of microsporocytes																	
Pro-II	346	221	504	81	52	146	335	263	77	103	105	58	14	107	41 to 45	48	73
T-II	149	23	75	154	360	372	387	269	279	222	261	325	267	102	225	147	247

[1] Meiosis I includes prophase through telophase-I (or interphase-I) and half of the microsporocytes in transition between telophase-I and prophase-II; meiosis II includes prophase-II through interphase-II and half of the microsporocytes in transition between telophase-I and prophase-II.

purpose these stages were defined as follows: At late anaphase the chromosomes at the poles form a rounded, compact group; at telophase these chromosomes begin to exhibit irregular outlines; in prophase-II the X-shaped chromosomes have separated somewhat and the nucleolus is visible. Lagging chromosomes decrease in frequency from late anaphase-I to early telophase-I. The frequencies of lagging chromosomes at prophase-II and late anaphase-I are not precisely correlated, but there is a certain relationship of high and low lagging chromosome frequencies at these stages. The variation in lagging chromosome frequency at prophase-II (0.53 to 1.74) is not related to the age of these anthers. High frequencies of lagging chromosomes were observed in anthers with meiotic ratios of 88:1 to 1:3 and low numbers of lagging chromosomes in anthers with 17:1 to 1:4 ratios (Table 2).

Table 2. *Frequencies of lagging chromosomes at late anaphase-I, early telophase-I, and prophase-II in two-nucleate cells only*

	Plant, floret, anther number	Frequency per cell of lagging chromosomes (no. lagging chromosomes/no. cells)			Ratio meiosis I:II	Frequency of multinucleate microsporocytes at Pro-II
		Late A-I	T-I	Pro-II		
A	2—1, 6, b	26/15 = 1.73		52/60 = 0.87	1:3	0.22
B	2—5, 1, a			46/48 = 0.96	1:1	0.18
C	2—3, 2, b			33/53 = 0.62	2:1	0.17
D	1—1, 1, b			21/19 = 1.11	1:10	0.17
E	1—1, 7, c			51/59 = 0.86	1:26	0.16
F	2—1, 6, c			44/45 = 0.98	1:2	0.15
G	1—1, 4, a			90/91 = 0.99	1:13	0.14
H	2—3, 2, c			106/127 = 0.84	1:2	0.11
I	2—3, 5, b			173/155 = 1.12	1:1	0.11
J	2—1, 1, a	35.5/15 = 2.37	15.5/32 = 0.50	79/72 = 1.10	1:2	0.10
K	1—1, 7, a			50/96 = 0.53	1:4	0.09
L	2—3, 5, c			147/151 = 0.97	2:1	0.06
M	2—3, 8, a			351/219 = 1.60	1:1	0.04
N	2—3, 8, c			208/176 = 1.18	1:1	0.03
O	2—1, 2, b	254/79 = 3.22		93/109 = 0.85	6:1	0.03
P	1—1, 9, a			53/47 = 1.13	1:22	0.02
Q	2—3, 8, b			248/187 = 1.33	2:1	0.01
R	2—3, 7, a	372.5/105 = 3.55	54/58 = 0.93	781/490 = 1.59	2:1	0.01
S	2—1, 4, a	157/52 = 3.02	20/43 = 0.47	193/260 = 0.74	1:3	0.01
T	1—1, 8, a			158/101 = 1.56	1:3	0.00
U	1—1, 6, a	418.5/174 = 2.41	53.5/77 = 0.69	202/221 = 0.91	1:1	0.00
V	2—3, 1, b			251/183 = 1.37	5:1	0.00
W	2—1, 3, a	173.5/36 = 4.82	32/39 = 0.82	87/50 = 1.74	15:1	0.00
X	2—1, 2, c	123/81 = 1.52		16/28 = 0.57	17:1	0.00
Y	1—1, 5, a	295/51 = 5.78	55/46 = 1.20	34/20 = 1.70	88:1	0.00

At A-I and T-I lagging chromosomes were tabulated as 1 (chromosome) or $^1/_2$ (chromatid); at Pro-II a chromosome, chromatid, or (rarely) a fragment was tabulated as 1 chromosome; the A-I and T-I figures are not, therefore, directly comparable with those of Pro-II.

Multipolar and Multinucleate Microsporocytes

Metaphase-I. An occasional tripolar spindle and an extremely rare quadripolar spindle is observed. The spindle poles are typically well separated, as shown in Fig. 29. In this tripolar configuration a group of bivalents and univalents is present at each of the three equators and some univalents are near the poles.

Anaphase-I. A few tripolar configurations are present (Figs. 2, 19; in Fig. 2 there may be only two spindles present). One of the three nuclei is nearly always considerably smaller than the other two. A 4-polar anaphase is shown in Fig. 30; lagging chromosomes lie between the four poles. Rarely scattered groups of chromosomes are observed at late anaphase.

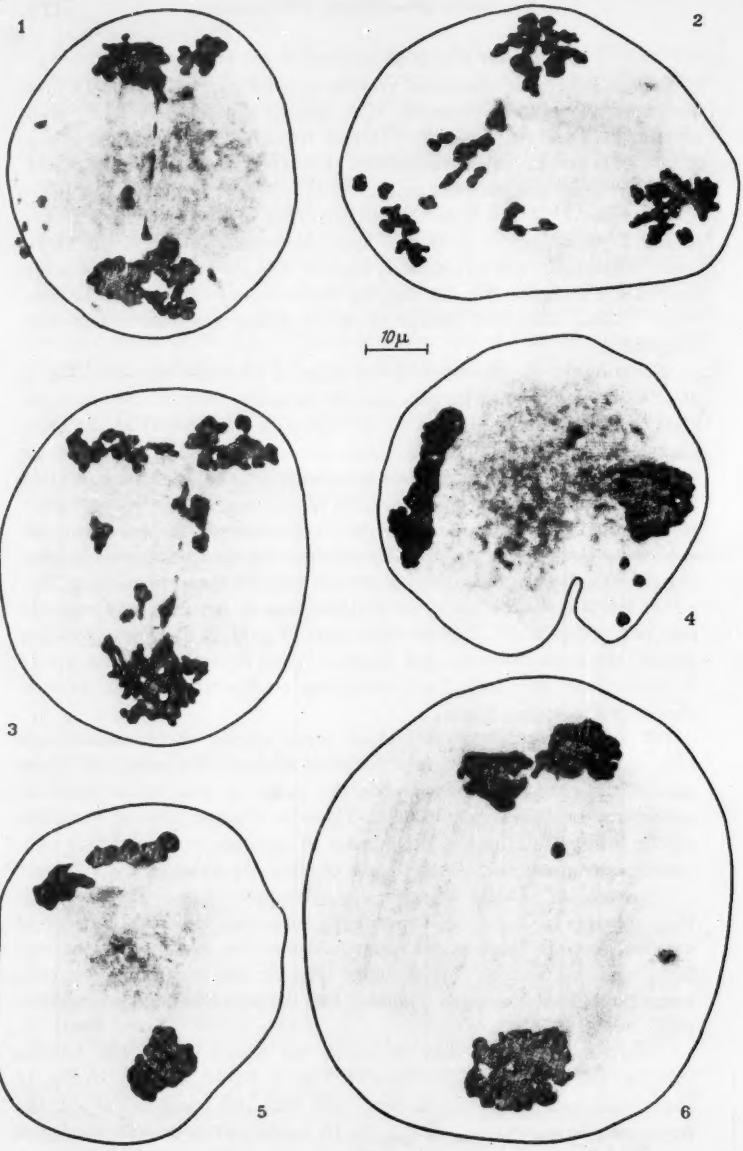
No example was observed at the stage of anaphase shown in Fig. 2 of a third pole occupied by only one chromosome. But at late anaphase a single chromosome (or even in one instance a fragment) at the periphery of the equator is occasionally connected to the polar nuclei by apparently separate phragmoplast bundles (Figs. 12, 31). In one cell two large nuclei and two chromosomes seem to be connected by five phragmoplasts (Fig. 33). In an occasional late anaphase cell the phragmoplast appears to bend at the peripheral point near the equator where a lagging chromosome lies; rarely a bend is present with no chromosome (Fig. 32).

At late anaphase a group of chromosomes at one pole may separate into two groups which lie somewhat apart (Figs. 1, 3, 5). The separating groups are most often unequal in size. When separation of the groups is incomplete, telophase-I or interphase configurations such as that shown in Fig. 4 may result.

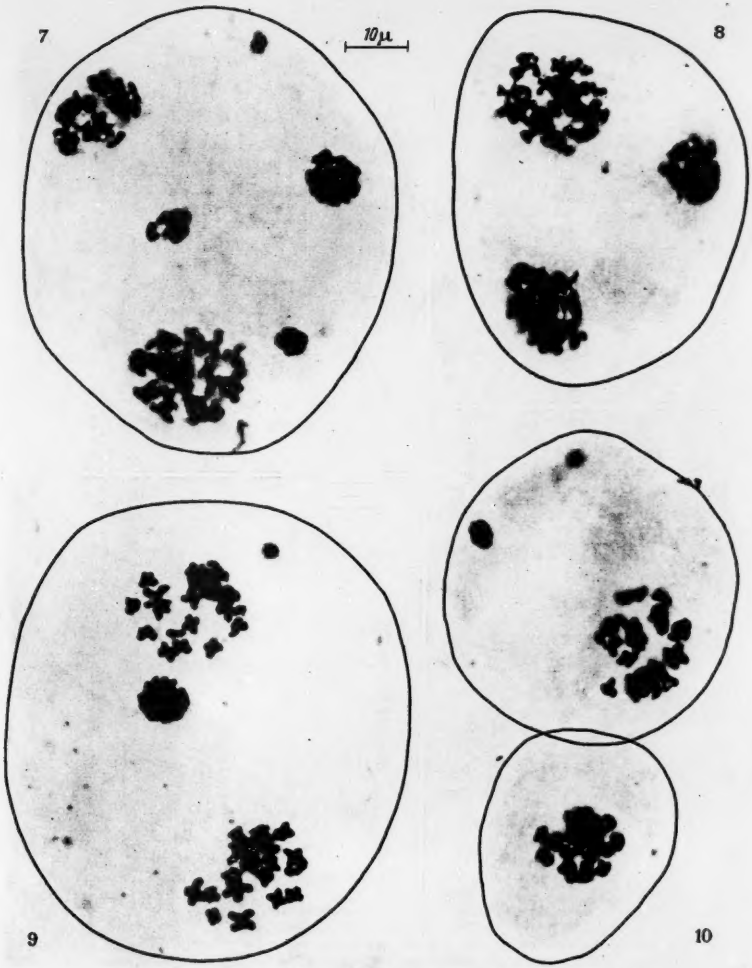
At late anaphase-early telophase some lagging chromosomes have two chromatid arms strongly attenuated towards the poles, and these apparently are stretching towards the poles by activity of terminal neocentromeres (WALTERS 1952). There is a great deal of variation among different anthers in the number of chromosomes exhibiting neocentric movement and in the degree of their attenuation.

Prophase-II. At this stage the chromosomes are somewhat separated; they are usually X-shaped, occasionally rods; two to several nucleoli of various sizes are present and often nucleolar-like material on the surfaces connects various chromosomes (Fig. 7); the surrounding nuclear membrane is not visible as a whole. Cell division has not always taken place (see p. 187).

Configurations that may represent the separation of one nucleus into two are observed at prophase-II (Figs. 6, 14, 15, 34, 39). In Fig. 14 the upper nucleus appears to have split into two equal nuclei and the lower may be separating. In Fig. 15, 15 a note particularly the nucleolus stretched between the two chromosome groups; the stretched nucleolus

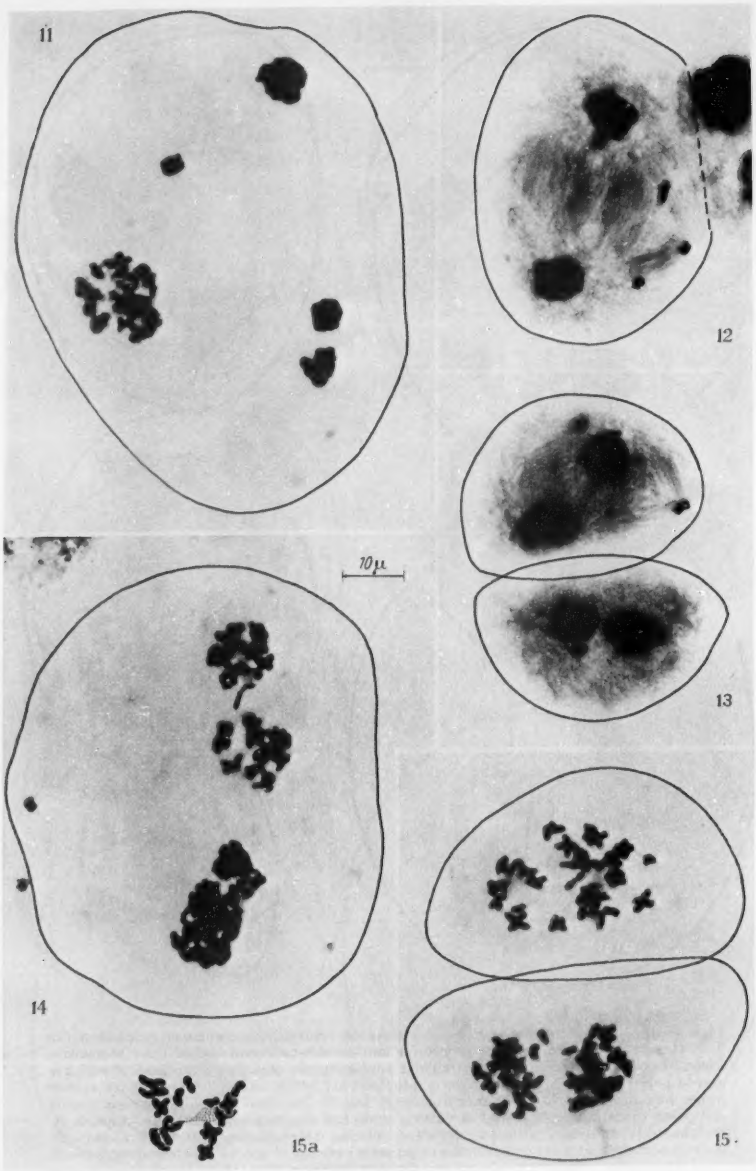


Figs. 1—6

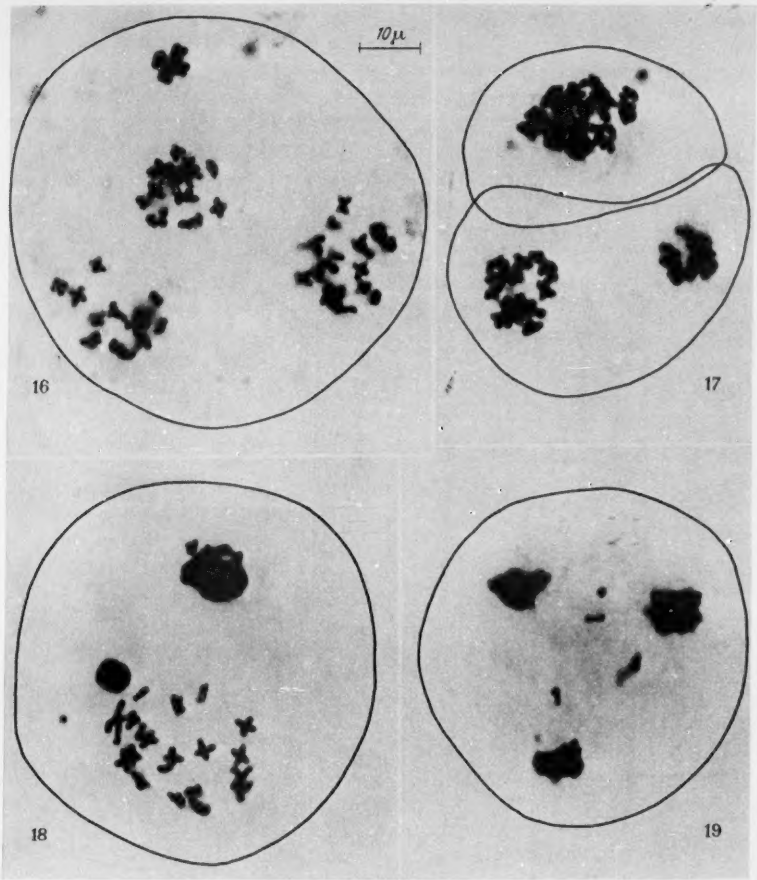


Figs. 7—10

Figs. 1—10. Microsporocytes of *B. trinii* × *carinatus*; cell walls are outlined in ink. Figs. 1, 3, 5. Late anaphase-I showing separation of two groups of chromosomes at the upper poles. Fig. 2. Late anaphase-I, showing a tripolar configuration. Fig. 4. Telophase-I; showing a spread nucleus at the left. Fig. 6. Early prophase-II; two groups of chromosomes at the upper pole are connected by matrix strands. Fig. 7. Prophase-II; an undivided microsporocyte containing five nuclei of various sizes and one single chromosome. Figs. 8, 9. Prophase-II; undivided microsporocytes containing three nuclei. Fig. 10. Prophase-II; a dyad with two nuclei in one cell; the sister cells have the characteristic round form found with very delayed cell division (compare Figs. 15, 17)

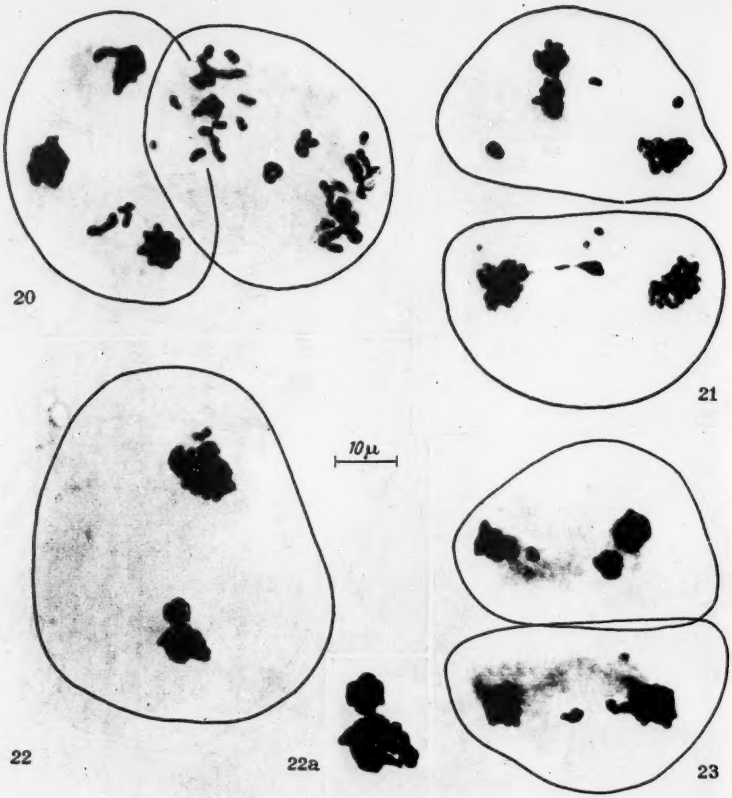


Figs. 11—15



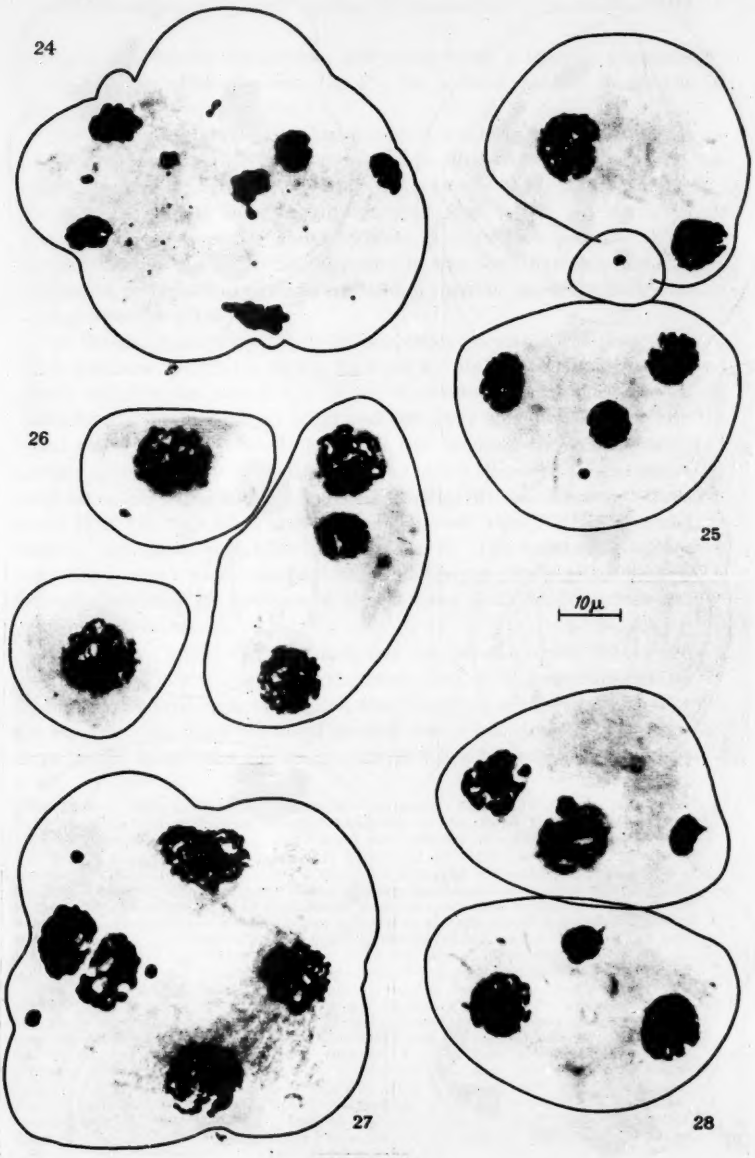
Figs. 16—19

Figs. 11—19. Microsporocytes of *B. trinii* × *carinatus*; cell walls are outlined in ink. Fig. 11. Prophase-II; an undivided microsporocyte containing four nuclei and one single chromosome. Fig. 12. Late anaphase-I; two separate phragmoplasts connect a single chromatid, lying at the periphery of the equator, to the two polar nuclei (see Fig. 31). Fig. 13. Late anaphase-II dyad; in the upper cell two chromatids lying at the periphery of the equator are connected to the polar nuclei by two separate phragmoplasts (see Fig. 35) Fig. 14. Prophase-II; an undivided microsporocyte containing three nuclei; the two upper nuclei are connected by a chromatid and matrix strands; the lower nucleus may be separating into two. Fig. 15. Prophase-II dyad; in the lower cell two groups of chromosomes are connected by a nucleolus stretched out between the right hand group and one chromatid in the left hand group (see Fig. 15 a). Fig. 16. Prophase-II; an undivided microsporocyte containing 4 nuclei, three nearly equal and one much smaller in size. Fig. 17. Prophase-II dyad with 2—1 nucleus distribution. Fig. 18. Prophase-metaphase-II; an undivided microsporocyte containing three nuclei; the lower nucleus is in prophase, and the two upper nuclei show typical pro-metaphase condensation. Fig. 19. Late anaphase-I, showing typical tripolar arrangement of nuclei

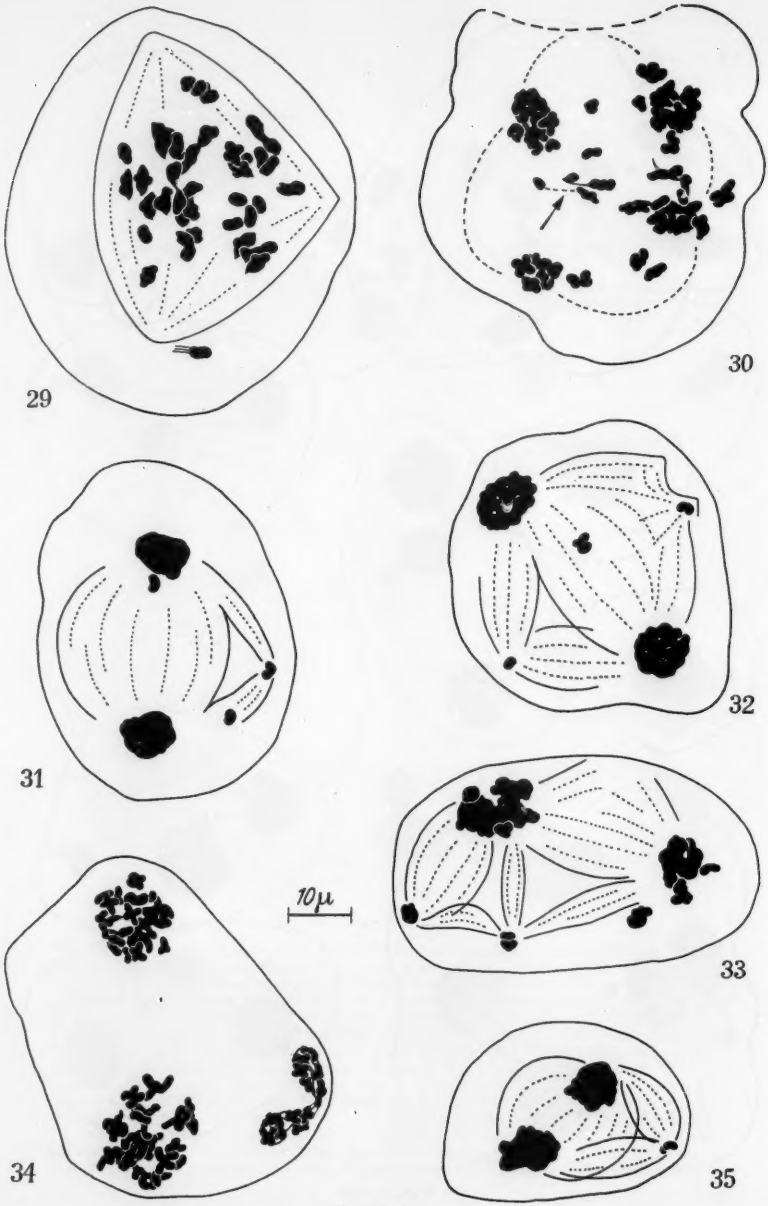


Figs. 20—23

Figs. 20—28. Microsporocytes in *B. trinii* × *carinatus*; cell walls are outlined in ink. Fig. 20. Anaphase-II dyad; the tripolar cell is in late anaphase, the bipolar cell in mid-anaphase. Fig. 21. Telophase-II dyad containing 5 nuclei; the two parallel nuclei in the upper cell are connected by fine matrix strands. Fig. 22. One cell of a late anaphase-II dyad; a small nucleus is barely attached to the lower nucleus (shown in Fig. 22 a at higher magnification). Fig. 23. Late anaphase-II dyad containing 5 nuclei; in the upper cell the small third nucleus is very close to the larger nucleus. Fig. 24. Late anaphase-II; an undivided cell containing six nuclei; note the cell wall bulges between nuclei. Fig. 25. Telophase-II dyad containing five nuclei (plus small cell with a chromatid or fragment); the five nuclei have 2—3 distribution. Fig. 26. Late telophase-II dyad-tetrad; the three nucleate cell is still single. Fig. 27. Late telophase-II; an undivided microsporocyte containing five nuclei; note close positions of the two nuclei at the left; note the pronounced cell wall bulges between nuclei. Fig. 28. Late telophase-II dyad containing six nuclei in 3—3 distribution; note the different positions, relative to the large nuclei, of the third small, condensed nucleus



Figs. 24—28



Figs. 29—35

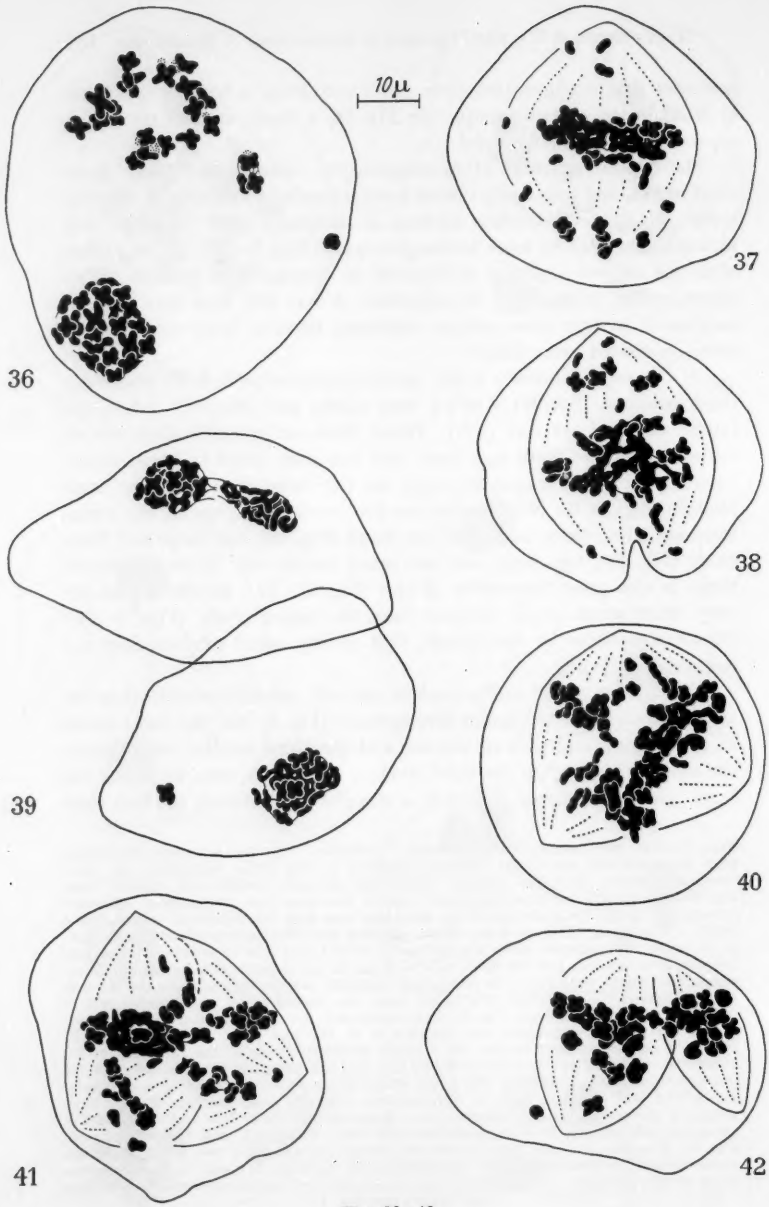
indicates this configuration does not result from a tripolar anaphase or from fusion of two groups. In Fig. 34, a third, smaller nucleus is separating into 2 equal nuclei.

The typical prophase-II microsporocyte contains two fairly equal sized nuclei, and frequently one or more separate chromatids or chromosomes. A single restitution nucleus is extremely rare. Three to five nuclei are present in some microsporocytes (Figs. 7—11, 16, 36), often with one or two separate chromatids or chromosomes present. (Two chromosomes occasionally lie together; it was felt that this may be accidental, so only those groups containing three or more chromosomes were designated extra nuclei).

Of the supernumerary types, microsporocytes with three nuclei are most common (249/281 = 89%), four nuclei are relatively infrequent (10%) and five are rare (1%). When three are present, there are all variations in size from two large and one very small to three nearly equal nuclei; most frequently there are two large nuclei and one small nucleus (Figs. 8, 9). Where there are four nuclei there are all size variations including three large and one small (Fig. 16), one large and three small (Fig. 11), two large and two small nuclei, etc. With five nuclei there is also great variability in size (Fig. 7). The smaller nuclei are very often much more compact than the larger nuclei (Figs. 9—11). It can sometimes be determined that a very small nucleus does not contain a nucleolus.

When three nuclei are present in one cell, most frequently they are well separated in a triangular arrangement (Fig. 8), the two large nuclei at the longitudinal ends of the cell and the third smaller nucleus near the equator; less often the third nucleus lies rather close to one of the large nuclei, sometimes almost in a straight line between the two large

Figs. 29—35. Microsporocytes of *B. trinii* × *carinatus*. Fig. 29. A tripolar metaphase with bivalents and univalents oriented primarily at the three equators; one chromosome, separate from the spindle, has fibers directed towards the spindle pole. Fig. 30. A quadripolar late anaphase-I; arrow indicates two prematurely separated chromatids; in the lower chromatid the short and long arms are separated, probably as a result of a break in the centromere region resulting from terminal neocentric movement; in the upper chromatid the short arm appears to move to the pole by centric or interstitial neocentric movement, and the lower arm to move to the opposite pole by terminal neocentric movement, resulting in an attenuated (probably centric) region. Note the cell wall bulges between the four poles (the upper bulge was pushed in by an adjoining cell, as indicated by the broken line). Fig. 31. Late anaphase-I; a single chromatid lies at the apex of two separate phragmoplasts that connect it to the two polar nuclei (see Fig. 12). Fig. 32. Late anaphase-I; on the left a single chromatid lying at the periphery of the equator is connected to the polar nuclei by two separate phragmoplasts; on the right the large phragmoplast connecting the polar nuclei forms an apex related to a chromatid and above that an apex with no chromosome. Fig. 33. Late anaphase-I; two large groups of chromosomes are connected by a large phragmoplast; four additional smaller phragmoplasts connect 2—3 chromosomes with each other and with the large nuclei. Fig. 34. Prophase-II; two large nuclei are present; at the right two small nuclei are connected by fine strands. Fig. 35. One cell of a late anaphase-II dyad; two chromosomes lying at the periphery of the equator are connected by separate phragmoplasts to the polar nuclei (see Fig. 13)



Figs. 36—42

nuclei (Fig. 9). Four or five nuclei may be well spaced or sometimes two lie rather close together (Figs. 7, 16; or 11).

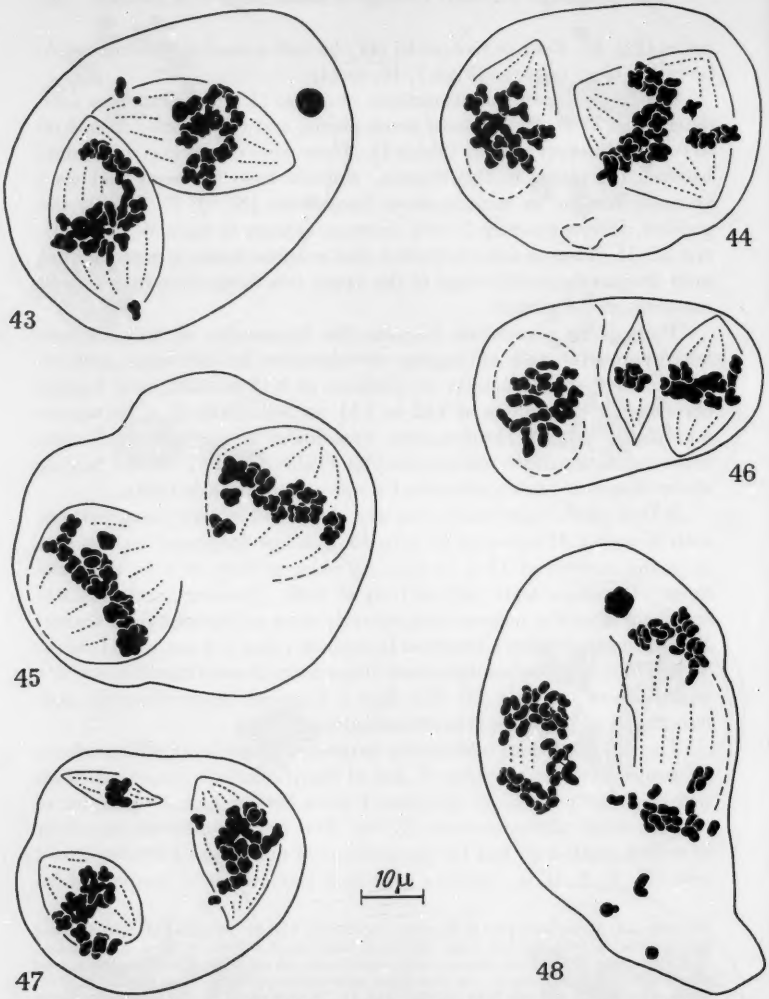
The frequencies of multinucleate prophase-II microsporocytes were determined in 41 anthers from seven plants, and these varied from 0.00 to 0.36 in different anthers (Table 1). There were no obvious differences between the plants in this respect. Anthers from the same bud were generally similar in multinucleate frequencies (N, Q, R; K, S); the greatest differences were 7—8% between anthers in buds W, AA, DD, and CC, II. There is some indication that multinucleates occur somewhat more frequently at this stage in the upper two-thirds than in the lower one-third of the anther.

There is no correlation between the frequencies of multinucleate microsporocytes and of lagging chromosomes in the same anthers. Anthers with multinucleate proportions of 0.13 to 0.22 have lagging chromosome frequencies of 0.62 to 1.11 per cell (Table 2). The highest numbers of lagging chromosomes were found in anthers which were without multinucleate microsporocytes (Table 2, T, W, Y) and lagging chromosomes in such anthers had a wide range (1.74 to 0.57).

Multinucleate microsporocytes were not found in very young anthers, with meiosis-I:II ratios of 88:1 to 15:1; a low frequency was present in young anthers of 13:1 to 5:1. In older anthers, 3:1 to 1:26, the range of multinucleate cells is 0.00 to 0.36. However, multinucleate frequency does not increase progressively with increasing age of anther. No multinucleates were observed in some 1:1 and 1:3 anthers (Table 1, T, BB) and very low multinucleate frequencies in certain additional late anthers from 1:1 to 1:22. The four 1:2 anthers have relatively high frequencies of prophase-II multinucleates.

There is no obvious relationship between frequencies of multinucleate microsporocytes at prophase-II and of specific meiotic stages. Anthers with high proportions of prophase-I have low or high frequencies of multinucleate microsporocytes (E, O). Few or no multinucleates occur in anthers with high and low proportions of metaphase-I or anaphase-I cells (G, T; Z, HH). Anthers with high proportions of anaphase-telo-

Figs. 36—42. Microsporocytes of *B. trinii* × *carinatus*. Fig. 36. Prophase-II; an undivided microsporocyte contains two large nuclei, a third small nucleus of three chromosomes, and a separate single chromosome; many small nucleoli are present, indicated by dotted areas. Fig. 37. Metaphase-II; an undivided microsporocyte containing the full chromosome complement and one large spindle. Fig. 38. Metaphase-II; a single microsporocyte containing the entire chromosome complement and one spindle; the lower pole of the spindle is bisected by beginning (very delayed) division of the microsporocyte. Fig. 39. Prophase-II; in the upper cell of this dyad two nuclei are connected by fine strands. Fig. 40. Metaphase-II; an undivided microsporocyte containing a single tripolar spindle; the chromosomes lie primarily in the equatorial regions. Fig. 41. Metaphase-II; an undivided microsporocyte with a single, possibly quadripolar spindle (the fourth pole hidden by the others); note one large and 3 small groups of chromosomes, aligned in equatorial regions between the presumed four poles. Fig. 42. Metaphase-II; an undivided microsporocyte with two spindles that appear to have one common pole, i.e., a branched spindle



Figs. 43—48. Microsporocytes of *B. trinii* × *carinatus*. Fig. 43. Metaphase-II; an undivided microsporocyte containing two spindles (or less probably one branched spindle) and a separate compact group of chromosomes with no spindle. Fig. 44. Metaphase-II; a single microsporocyte containing one bipolar and one tripolar spindle; very delayed cell division is beginning, indicated by the brokeen line near the bottom. Fig. 45. Metaphase-II; an undivided microsporocyte containing two separate spindles. Fig. 46. Metaphase-II; a partially divided (indicated by broken line) microsporocyte; the cell at the right contains two spindles of different sizes and with different numbers of chromosomes; in the cell at the left the presence of a spindle is not certain. Fig. 47. Metaphase-II; an undivided microsporocyte containing two large and one small spindles. Fig. 48. Anaphase-II; an undivided microsporocyte containing two unconnected separating groups of chromosomes; at the top a compact clump of chromosomes shows no evidence of a spindle or of anaphase separation; at the bottom three separate chromosomes are unrelated to the anaphase groups

phase-II and tetrads have low or high frequencies of multinucleate microsporocytes (BB, EE). While multinucleate microsporocytes were absent or infrequent in anthers with unusually high proportions of telophase-I and prophase-II cells (M, S, T, U, Z), equally few were observed in some old anthers with low proportions of these stages (R, BB, HH). However, anthers with 10% or more multinucleates at prophase-II do have relatively low proportions of telophase-I and prophase-II microsporocytes. It may also be significant that prophase-II multinucleate frequencies were relatively high in anthers containing numerous microsporocytes in prophase-I and anaphase-II to tetrads, and few in the intervening stages.

Metaphase-II. At the end of prophase-II the nucleoli have disappeared, the nuclear membrane is gone, and the chromosomes are widespread (Fig. 18, bottom nucleus). Cell division has not always taken place (see p. 187). At pro-metaphase-II the chromosomes form a very condensed round group that cannot be flattened or spread (Fig. 18, top and left nuclei); if a dyad is formed, the pro-metaphase group lies in the center of each cell. At metaphase the chromosomes separate somewhat and line up on the equatorial plate; often some chromosomes are scattered towards the poles.

Tripolar spindles were occasionally observed in one cell of a dyad; more complex configurations found in undivided cells are described on p. 190, 191. Two groups of chromosomes are occasionally found in one cell of a dyad. It can sometimes be established that each chromosome group occupies a spindle (Fig. 46) or that one of the two groups definitely has no spindle.

Anaphase and Telophase-II. Cell division is sometimes still lacking or incomplete. Spindles and chromosome distributions in such microsporocytes are described on p. 190, 191.

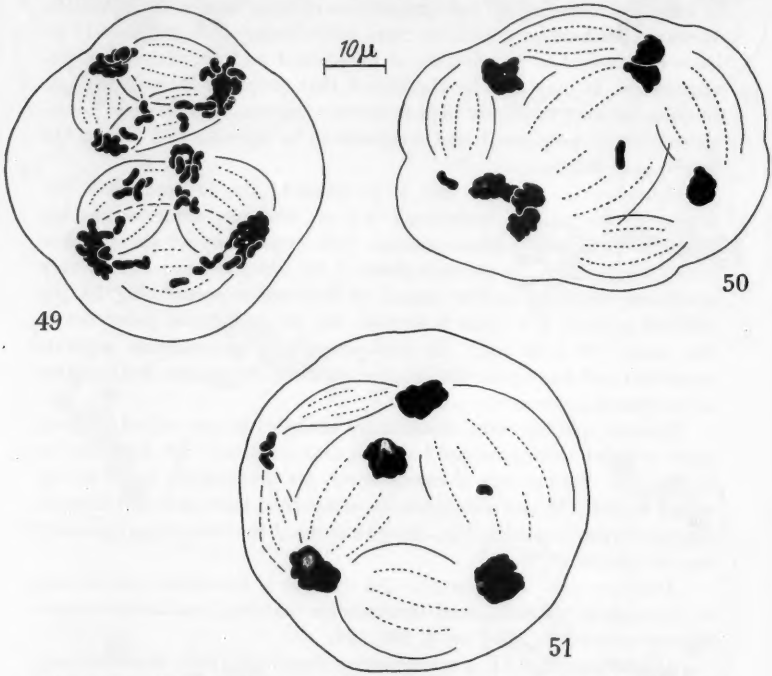
At late anaphase-II, a configuration described above is occasionally found; i.e., a chromosome lies at the periphery of the equator, and the phragmoplast is bent at that point, or separate phragmoplast bundles extend from that chromosome to the poles (Figs. 13, 35).

When three nuclei are present at late anaphase-II, they may be well spread in a triangular arrangement (Fig. 20), or a smaller nucleus lies close to one of the two large nuclei (Fig. 23). Splitting of one nucleus into two is believed to occur at this stage (Figs. 21, 22).

At late anaphase-telophase-II the dyad most frequently contains four nuclei, about equal in size. The number of nuclei may, however, vary from 2 to 7 (Figs. 24, 25, 27, 28, 50), with 5 by far the most frequent aberrant number.

As before, three chromosomes together were called a nucleus; most extra nuclei, however, contained more than three chromosomes. When

five nuclei are present, one is nearly always considerably smaller. When there are six, there are sometimes 4 large and 2 small nuclei (Fig. 28); or various sizes of nuclei are present. The smaller nuclei are frequently more condensed than the larger ones (Fig. 28). The smaller nucleus may



Figs. 49—51. Microsporocytes of *B. trinii* × *carinatus*. Fig. 49. Late anaphase-II; an undivided microsporocyte containing two unconnected groups of chromosomes; each group has a tripolar distribution. Fig. 50. Late anaphase-II; an undivided microsporocyte containing 7 nuclei connected by 5 phragmoplasts; note cell wall bulges between nuclei. Fig. 51. Late anaphase-II; an undivided microsporocyte containing four nuclei connected by 3 phragmoplasts; at the left two chromosomes are connected by a small phragmoplast to an upper nucleus, and possibly by another small phragmoplast to a lower nucleus

form a triangular or straight line arrangement with the other two (Figs. 25, 26, 28).

The frequency of telophase-II microsporocytes with supernumerary nuclei varied among this sample of 41 anthers from 0.00 to 0.40 (Table 1). No appreciable differences in multinucleate frequencies were found among anthers from one floret. There may be a tendency for higher proportions of multinucleate microsporocytes to occur in the bottom third of individual anthers.

Most frequently individual anthers had about the same proportions of multinucleate microsporocytes at prophase-II and telophase-II. In some instances, however, multinucleate frequencies were very much lower at telophase-II (O, AA, EE), and in a few anthers multinucleate frequencies were very much higher at telophase-II than at prophase-II (L, FF, II).

Few or no multinucleate telophase-II microsporocytes were found in young anthers containing relatively few cells in this stage; i.e., C, H, J. The highest frequencies occurred in old anthers; i.e., FF (1:13), II (1:26); and also in anthers CC (1:4) and GG (1:19). However, a high frequency of multinucleates was also present in the younger anther L (2:1); and they were relatively infrequent in such old anthers as BB (1:3), EE (1:8) and HH (1:22). In two anthers with very similar proportions of cells in anaphase-telophase-II and tetrads (FF, HH) the telophase-II multinucleate frequencies were 0.40 and 0.06[1].

Microsporocyte Division. In *Bromus* the microsporocyte normally divides into a dyad during telophase-I and early prophase-II, and into a tetrad at late telophase-II. In this hybrid dyad division may be complete at telophase-I or delayed through telophase-II; in the latter event the single cells divide into tetrads at very late telophase-II.

Variation occurs within individual anthers in rate of microsporocyte division; thus in some anthers microsporocytes are divided at prophase-II while in others microsporocytes remain single cells at telophase-II. In anthers in which all prophase-II microsporocytes are divided, all subsequent stages are normally divided. In anthers in which dyad division is not generally accomplished by prophase-II, division continues to occur, so that fewer undivided cells are found at later stages than at prophase-II. However, some cells remain partially or completely undivided in anthers in which a high proportion of prophase-II cells were undivided.

Extreme variations are found among individual anthers in the rate of microsporocyte division, expressed by the proportion of microsporocytes partially or completely divided at prophase-II (Table 3): from 0 to 100% of the microsporocytes in individual anthers are divided at prophase-II. Anthers of the same floret are more often similar, but wide differences may occur, e.g., between (Table 3) E, K and J, BB. There is a possible relationship between rate of cell division and age of anther. Cell division tends to occur earlier in young anthers, although there are some notable exceptions (see Table 3, 88:1 to 2:1). Moreover,

[1] It will be shown below that division of multinucleate microsporocytes is delayed. Thus some accumulation of multinucleate dyads occurs in older anthers. However, the fact that frequencies of multinucleate dyads may be very low in very old anthers indicates that this does not account entirely for differences between anthers in multinucleate frequency at telophase-II.

Table 3. *A comparison of the frequencies of partially-to-completely divided binucleate and multinucleate microsporocytes at prophase-II in individual anthers; and a comparison of the frequencies of binucleate microsporocytes in anthers of various ages*

	Plant, floret, anther number	Ratio meiosis I:II	Total no. Pro-II binucleate microsporocytes	Total no. Pro-II multinucleate microsporocytes	Per cent microsporocytes partially-completely divided at Pro-II	
					Binucleate	Multinucleate
A	1—1, 5,a	88:1	20	0	100	—
B	2—4, 1,a	59:1	14	0	86	—
C	2—5, 2,a	23:1	42	0	100	—
D	2—5, 2,c	22:1	42	0	100	—
E	2—1, 2,c	17:1	38	0	0	—
F	2—1, 3,a	15:1	50	0	100	—
G	2—5, 2,b	15:1	44	0	100	—
H	2—3, 9,a	13:1	72	1	53	0
I	2—3, 6,a	11:1	88	2	32	50
J	2—3, 1,a	7:1	136	3	99	100
K	2—1, 2,b	6:1	110	3	42	0
L	2—3, 1,b	5:1	184	0	92	—
M	2—4, 2,a	3:1	91	7	86	29
N	2—1, 7,b	3:1	202	3	55	0
O	2—3, 2,b	2:1	54	11	44	0
P	2—3, 5,c	2:1	152	9	61	56
Q	2—3, 7,a	2:1	491	3	90	100
R	2—3, 8,b	2:1	189	2	87	100
S	2—5, 1,a	1:1	50	11	86	55
T	1—1, 6,a	1:1	221	0	96	—
U	1—1, 11,a	1:1	491	13	90	23
V	2—1, 7,a	1:1	339	4	73	75
W	2—3, 5,b	1:1	157	18	73	44
X	2—3, 8,a	1:1	221	10	72	60
Y	2—3, 8,c	1:1	174	6	57	50
Z	2—1, 1,a	1:2	73	8	60	37
AA	2—1, 6,c	1:2	37	8	57	37
BB	2—3, 2,c	1:2	130	16	76	25
CC	1—2, 1,a	1:2	274	50	80	28
DD	2—1, 4,a	1:3	261	2	82	100
EE	2—1, 6,b	1:3	60	17	48	17
FF	1—1, 8,a	1:3	99	0	94	—
GG	1—1, 7,a	1:4	96	9	71	11
HH	2—1, 6,a	1:4	49	8	65	0
II	2—2, 2,a	1:8	9	5	0	0
JJ	1—1, 1,b	1:10	19	4	32	0
KK	1—1, 4,a	1:13	93	15	84	47
LL	1—1, 8,a	1:22	47	1	66	100
MM	1—1, 7,c	1:26	61	12	84	17
Total			4980	261		

in only one of 14 older anthers (1:2 to 1:26) were more than 90% of prophase-II microsporocytes divided.

The rates of cell division varied significantly in different regions of some anthers, but there was no correlation between any one region and a greater or lesser amount of cell division. That is, the highest propor-

tion of divided prophase-II microsporocytes occurred in the bottom third of some anthers, and the top or middle third of other anthers.

The course of normal division of a microsporocyte at telophase-I or early prophase-II is shown in Fig. 52, A—D. A faint, thin, clear line appears at the equator in the center of the cell; it becomes more distinct and extends to the edges. As the new cell wall forms on either side, the clear space between them widens. Sometimes the cell lies at such an angle that the new walls appear as separate curved lines. Formation of the new walls continues (sometimes bands connect regions of the two new cells) until finally the daughter cells are completely separate. The new cells are somewhat flat on one side, and elongate (Figs. 15, 17).

When cell division is delayed (Fig. 52, a—d), the clear space at the equator may widen until it looks almost like a hole, before eventually wall formation progresses to the edges of the cell. Often the new walls are formed from one edge towards the center of the cell, and the microsporocyte is divided only on one side. Sometimes wall formation begins on opposite sides of the cell and the center remains undivided for some time. When division is very long delayed at prophase-II the resulting daughter cells tend to be round (Fig. 10).

When a third cell is cut off around a lagging chromatid or extra nucleus, these new cell walls form a little later than the primary walls between the two major nuclei.

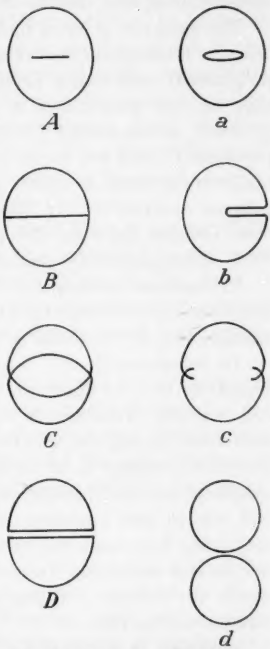


Fig. 52. Diagrammatic representations of microsporocyte divisions. *A*—*D* normal division; *a*—*d* delayed division

At telophase-II the dyad divides into a tetrad. Occasionally one cell divides ahead of the other (Fig. 26); such a microsporocyte will be called a "dyad-tetrad". When extra nuclei are present, an equivalent number of cells may be formed, or two nuclei are included in one cell. When single microsporocytes divide at telophase-II, they usually divide very irregularly.

The presence of lagging chromosomes is to some extent correlated with delayed microsporocyte division. In 377 prophase-II microsporocytes in which the dyad division was complete, there was an average

of 0.75 chromosomes[1] lying outside the nuclei; in 1146 prophase-II microsporocytes with no indication of division, an average of 1.04 free chromosomes was present. These free chromosomes were distributed throughout the cells, not necessarily located at the equator. In 498 telophase-II dyad-tetrads, the single cell contained an average of 1.65 chromosomes, the two divided cells an average of 1.12 chromosomes.

The presence of extra nuclei is correlated with a much more striking delay in microsporocyte division. The proportions of divided binucleate prophase-II cells varies among individual anthers; but whatever these may be, the proportions of 3—5 nucleate cells that are divided are generally much smaller (Table 3); e.g., in anther U, 90% of binucleate prophase-II cells are divided but only 23% of the cells with 3 or more nuclei are divided, in anther BB 76% binucleate and 25% multinucleate cells are divided, etc. In total, 79% of 4980 prophase-II binucleate cells were divided, 35% of 230 prophase-II trinucleate cells, and 13% of 31 4—5-nucleate cells were divided.

Trinucleate microsporocytes only rarely divide into three cells at prophase-II or metaphase-II. Generally two nuclei, one of these the smaller one, are included in one cell (Fig. 17).

In telophase-II, 271 5-nucleate dyad-tetrad microsporocytes were found; in 95% of these the extra nucleus was contained in the single cell (Fig. 26). This cannot be entirely attributed to a delay in anaphase movement in tripolar spindles. In nearly all bi- and tripolar dyads the five nuclei appear to be in the same stage of meiosis. In 13 of 18 late anaphase and early telophase dyads in which they differed, the tripolar cell was in late anaphase and the bipolar in early telophase; in the remaining five dyads the tripolar was in early telophase and the bipolar cell in late anaphase. In each of 13 additional mid- and late anaphase dyads the tripolar cell was in late anaphase and the bipolar in mid-anaphase (Fig. 20).

Spindles in Undivided Cells. Second metaphase dyads have two bipolar, parallel spindles; rarely one spindle is bipolar and one is tripolar. Various types of spindles are found in undivided metaphase-II cells and the late anaphase-telophase-II cells contain 2—7 nuclei. Spindle types and the chromosome distributions probably derived from them are: (1) A single bipolar spindle (Fig. 37) produces two large nuclei; if one pole of the metaphase spindle is split by a partial cell wall (Fig. 38), three nuclei are formed. (2) Two separate bipolar spindles (Fig. 45) produce four nuclei; if two poles are very close they may fuse (probably Fig. 43) and three nuclei result. (3) Three separate spindles (Fig. 47), one of which is smaller, form 4 large and 2 small nuclei. (4) Two separate

[1] The term "chromosome" here indicates a chromosome, chromatid, or rarely a fragment.

spindles, one bipolar and one tripolar (Fig. 44), form 5 nuclei (possibly Fig. 27). (5) A single tripolar configuration may have two or three spindles (Figs. 40, 42); in either event 3 nuclei form. (6) A rare configuration is a single 4-polar spindle (possibly Fig. 41) which will produce 4 nuclei. Two separate tripolar spindles have been found at anaphase-II (Fig. 49), and should form 6 nuclei (Fig. 24). More complex configurations were probably present at metaphase-II but could not be analyzed.

It is sometimes observed at metaphase-II or early anaphase-II that one group of chromosomes within a microsporocyte lacks a spindle and is apparently inert (Figs. 43, 48); note the compact arrangement of these chromosome groups. The separate, inert chromosome group will form a separate nucleus at late anaphase-telophase-II. Thus the configuration shown in Fig. 43 should result in 4 nuclei (assuming fusion of nuclei at the two close poles), that in Fig. 48 will form 5 nuclei.

As observed in meiosis-I, a single chromosome lying at the periphery of the equator of a late anaphase cell may be connected to the polar nuclei by apparently separate phragmoplasts (Figs. 13, 35); in a 4-nucleate single microsporocyte (Fig. 51), two chromosomes are connected to an upper nucleus by a separate, distinct phragmoplast and perhaps also to the lower nucleus.

In some microsporocytes that are single at second late anaphase or telophase, the cell wall bulges between two nuclei and is indented at the far side of each nucleus (Figs. 24, 27, 50); this was also observed in a 4-polar cell at late anaphase-I (Fig. 30). Because the cell is viewed in one plane, there may not be as many bulges visible as there are "pairs" of nuclei.

Discussion

Multipolar spindles have been produced in plants and animals by various experimental treatments, e.g., by chloral hydrate in *Vicia* (Sakamura 1920), ethyl-ether in *Daphne* (Yamaha 1927), cold in *Salix* (Ehrenberg and Östergren 1942), hydrostatic pressure in *Tradescantia* (Pease 1946), OMPA in *Allium* (Walsh and McMahon 1953). Multipolar or supernumerary spindles occur spontaneously in *Melandrium* (Heitz 1926), *Zea* (Beadle and McClintock 1928), *Triton* (Fankhauser 1934), haploid *Oryza* (Morinaga and Fukushima 1934), *Anisolabis* (Schrader 1941), inbred cattle (Knudsen 1958), and in human cancer cells (Therman and Timonen 1954), in interracial hybrids of *Drosophila* (Koller 1934), intergeneric hybrids of *Cairina* × *Anas* (Crew and Koller 1936), in species and interspecific hybrids of *Mentha* (Swanson and Nelson 1942), and in interspecific hybrids of *Bromus* (Walters 1958).

It has been suggested that in certain animal cells multipolar spindles result from a disturbance of the division cycles of centrioles and chromo-

somes such that extra centrioles are produced (THERMAN and TIMONEN 1954, SCHRADER 1941, KNUDSEN 1958). The asynchrony of centriole and chromosome reproduction has been related to a change in the relative duration of prophase and metaphase in human cancer cells — the relative duration of prophase is greatly reduced (THERMAN and TIMONEN 1954). The blocking of metaphase and subsequent increase in frequency of that stage has been related to the occurrence of multipolar spindles after cold treatment in *Triton* (BÖÖK 1945).

In plants the suggestion has also been made that asynchronous division of chromosomes and "pole determinants" or "spindle organizers" results in multipolar or supernumerary spindles (SWANSON and NELSON 1942, WALTERS 1958). The aberrant spindles occur in meiosis in plant materials in which large numbers of univalents are present (WALTERS 1958, BEADLE and MCCLINTOCK 1928), but also in those containing regularly paired chromosomes (HEITZ 1926, SWANSON and NELSON 1942). While it has been observed that the frequency of multipolar or multiple spindles varies within and between anthers (HEITZ 1926, BEADLE and MCCLINTOCK 1928, SWANSON and NELSON 1942, WALTERS 1958), their occurrence has not been related to alterations in duration of meiotic stages or to other simultaneously variable meiotic aberrations.

The present *Bromus* hybrid exhibits variation in frequency of multipolar configurations among individual anthers as well as variation in relative proportions of meiotic stages, in frequencies of lagging chromosomes, and in rate of cell plate formation. Before the relationships of these meiotic aberrations are discussed, it is desirable to consider the origin of multipolar spindles, supernumerary spindles, and multinucleate microsporocytes.

Multipolar spindles were previously observed in *Bromus rubens* × *carinatus* at meiotic metaphase and anaphase, and the suggestion was made that they arose from splitting or extra division of spindle organizers in first or second prophase (WALTERS 1958). In the present hybrid multipolar spindles have been observed at first and second metaphase and anaphase, and it may be suggested again that extra divisions of spindle organizers have occurred in the preceding prophases. However, it has also been observed that daughter chromosome groups may split at first or second late anaphase-early telophase or nuclei may split at prophase-II and the two portions are more or less separated. It is now proposed that under certain conditions evidently expressed in this particular hybrid, the spindle organizers can split or divide at any time during the meiotic cycle. Whether the split results in formation of a multipolar spindle or multinucleate microsporocyte may depend on the stage of meiosis when it occurs.

Similarities observed in other materials between centromeres and centrioles (POLLISTER and POLLISTER 1943) suggested the possibility that in the present hybrid some of the small prophase-II nuclei were formed from lagging chromosomes by attraction of their centromeres. However, the absence of any correlation between frequencies of extra nuclei and of lagging chromosomes opposes this suggestion. In this material, therefore, extra nuclei are not thought to arise as aggregates of separate chromosomes, but from subdivisions of existing nuclei, whose formation is attributed to the previous establishment of spindle poles.

At metaphase, the arrangement of the tripolar and quadripolar spindles is such that the poles tend to be well separated in the cell. A similar arrangement of nuclei was observed in the majority of trinucleate prophase-II microsporocytes. Illustrations of multipolar configurations in various materials often show the poles to be about equally spaced (HEITZ 1926, OKABE 1929, FANKHAUSER 1934, SCHRADER 1941). It has previously been suggested that centrosomes of *Anisolabis* may repel one another during spindle formation; at the completion of this movement the centrosomes of a quadripolar configuration are as far apart as the cell wall allows and the repelling forces between them are in equilibrium (SCHRADER 1941). It may be suggested that spindle organizers in *Bromus* also repel one another, most effectively during spindle formation, but to some extent also during other stages of meiosis; i.e., the separate entities resulting from the split or division of a spindle organizer repel one another. It is further suggested that the chromosomes are closely associated with the spindle organizers at prophase and late anaphase-telophase and follow their movement (WALTERS 1958), and it is possible that the numbers of chromosomes in the two groups indicate the relative sizes of the new spindle organizers. SCHRADER (1941) suggests the separation of sister centrosomes may be hindered if the spindle fibers are already established. In *Bromus* the location of spindle poles or nuclei in a given microsporocyte may depend upon the time of spindle organizer division. Well separated poles or nuclei at metaphase or anaphase may indicate a spindle organizer split in prophase (Fig. 53, A, B) or during the preceding meiotic division; adjacent poles or nuclei or chromosome groups may indicate a very recent spindle organizer split and possibly the hindering effect, after prophase, of an already formed spindle or phragmoplast (Fig. 53, a—c); well separated nuclei at prophase-II may indicate a spindle organizer split during prophase of the preceding division. Two portions of a nucleus that fail to separate sufficiently may be enclosed together in a common nuclear membrane.

When the anthers of the present hybrid have reached a certain stage of development, the range of meiotic stages increases markedly. It is,

therefore, assumed that the rate of meiosis is altered and variable among individual microsporocytes. In anthers with the same range of stages or with the same meiosis-I:II ratios, the proportions of microsporocytes in the various stages of meiosis may be very different. This may depend at least in part upon the age of the anther: in a young anther only a few microsporocytes have reached the final stages of meiosis whereas in an old anther few still remain in the beginning stages. In less extreme instances, in anthers of seemingly comparable ages, it is still not possible to determine whether differences in frequencies of comparable stages demonstrate corresponding differences in rates of these stages in individual anthers. A further alteration typical of some, usually older anthers, which suggests an alteration in the time span of meiosis, is the direct transition from early telophase-I (in one instance late anaphase-I) to prophase-II.

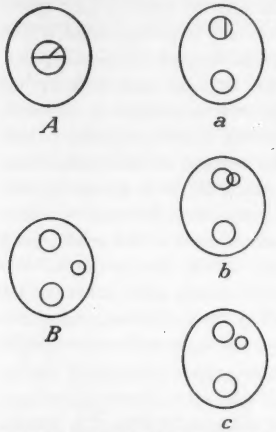


Fig. 53. Diagram showing locations of spindle poles or nuclei resulting from division of spindle organizers at different stages of meiosis. *A* the organizer splits at prophase; *B* the spindle poles or nuclei are well separated at metaphase or anaphase. *a* the organizer splits at anaphase-telophase; *b*, *c* poles or nuclei are close at these stages; *c* if the split occurs at anaphase-telophase-I or prophase-II, poles or nuclei are close at prophase-II

It has been observed that very young anthers, with meiotic ratios 88:1 to 5:1, contain no or very few multinucleate microsporocytes. Those anthers containing relatively high frequencies of multipolar spindles and multinucleate microsporocytes are generally older anthers, with meiotic ratios of 3:1 to 1:26. A relationship is therefore suggested between the irregular alteration in rate of meiosis characteristic of older anthers and the occurrence of irregularities of spindle organization that produce multipolar spindles and supernumerary nuclei. It is also noted, however, that an occasional old anther contains few or no multinucleate microsporocytes.

It has not been possible to determine that the relative frequency of any specific stage of meiosis is invariably or definitely related to the frequency of spindle irregularity. It is observed that the anthers in which high frequencies of prophase-II multinucleates are found have low proportions of microsporocytes in telophase-I and prophase-II; however, a low incidence of prophase-II multinucleates is also observed in some anthers with low proportions of microsporocytes in these stages. While older anthers with high proportions of anaphase-II to tetrad

microsporocytes generally have high frequencies of prophase-II multinucleates, some anthers of this type have very low frequencies of multinucleates. Similarly, while low frequencies of multinucleate telophase-II microsporocytes were found in young anthers containing relatively few cells in this stage, both high and low frequencies of telophase-II multinucleates were observed in older anthers with high proportions of anaphase-II to tetrad microsporocytes. It can only be said that generally the higher proportions of prophase-II multinucleate microsporocytes are found in anthers containing relatively few telophase-I and prophase-II cells, and the larger proportions of telophase-II multinucleate microsporocytes are found in anthers in which these late stages are relatively numerous. Because the frequencies of various stages may be determined in part by the age of individual anthers, it is not possible to suggest any correlation between duration of specific meiotic stages and spindle irregularity.

The number of multinucleate telophase-II microsporocytes depends upon the frequency of multipolar spindles and divisions of nuclei occurring in preceding stages. It has been determined that cell plate formation is very much delayed in multinucleate dyads, and so their transformation to tetrads is delayed. It has also been shown that accumulation of multinucleate dyads cannot completely account for the differences between anthers in multinucleate frequency at telophase-II. In some instances multinucleate frequency is very much lower at telophase-II than at prophase-II. In these anthers, when the cells now in telophase-II were passing through earlier stages of meiosis there was not as much multinucleate formation as there is now in stages preceding prophase-II. In some instances there is a very large increase in multinucleate frequency from prophase-II to telophase-II, so large that it cannot be attributed to accumulation of multinucleate dyads. Apparently in these anthers additional spindle irregularities occurring at metaphase- and anaphase-II were added to those already present at prophase-II. Multinucleate production, then, is not constant in individual anthers. Spindle aberrations may occur with different frequencies at different times in the same anther.

To summarize, the present observations suggest a relationship between an altered, irregular rate of meiosis and the spindle irregularities that produce multipolar spindles and multinucleate microsporocytes. The nature of the relationship is not clear. Evidence has been mentioned above that spindle aberrations are most often found at prophase-II when this stage is relatively infrequent, and at telophase-II when this stage is relatively abundant; but these are not constant correlations, and the possible effect of differences in ages of anthers precludes a suggestion of some relationship between duration of meiotic stage and

frequency of spindle irregularity. It can be demonstrated that frequencies of spindle aberrations are not necessarily constant in individual anthers, but may change during the course of the meiotic divisions, influenced by factors yet unknown.

The pronounced prometaphase contraction described in this hybrid has been observed by BAJER and MOLÈ-BAJER (1956) in various plant species, in which the forces appeared to act from all directions, or primarily from the poles, or sometimes from intermediate directions. BAJER and MOLÈ-BAJER suggest that prometaphase contraction may be due to the longitudinal orientation of spindle particles or to alterations in surface tension around the chromosomes following disappearance of the nuclear membrane. DARLINGTON (1937) attributes this clumping of chromosomes to a temporary reduction in chromosome repulsion; and SCHRADER (1941) suggests that in *Anisolabis* disintegration of the nuclear membrane probably also plays a role. In the present material the chromosomes appear either to be pushed from all directions or to be strongly attracted to one another, and it is observed that individual nuclei in a microsporocyte do not necessarily undergo the prometaphase contraction simultaneously (Fig. 18).

The movement of univalents to the poles and back to the equator at metaphase is not as pronounced in the present hybrid as it was in *B. rubens* × *carinatus* (WALTERS 1958), and these spindles do not elongate and curve as do those in *rubens* × *carinatus*. The much higher proportion of univalents in *rubens* × *carinatus* may cause a delayed metaphase, and consequently the elongated spindles and more pronounced movement of univalents in that hybrid. It is believed that some movement of univalents to the poles and back to the equator occurs generally at metaphase in *Bromus* hybrids.

It has been observed in other *Bromus* hybrids that lagging chromosome frequency decreases through late stages of anaphase, and anaphase was extended by definition in an attempt to include all chromosome movement to the poles. However, it is now observed that lagging chromosome frequency continues to decrease even between late anaphase (i.e., the chromosomes at the poles are in a rounded, compact group) and early telophase (i.e., chromosome outlines begin to be irregular). Thus chromosomes continue to reach the poles past the stage when movement was formerly thought to cease. The continued movement of chromosomes to poles at very late anaphase and early telophase has also been observed by ÖSTERGREN (1958) and BAJER (1958).

In the present hybrid there is no relation between lagging chromosome frequency and the age of the anther. Thus the rate or extent of chromosome movement is not influenced by the alterations in meiotic rates occurring in older anthers. If the differences in lagging chromo-

some frequency between anthers are not attributable to corresponding differences in frequencies of metaphase univalents, possibly the poles themselves may vary with respect to their relations to chromosome movement.

At very late anaphase the half-spindles are extremely short or absent, and a phragmoplast connects the two nuclei (BAJER 1951, DUNCAN and PERSIDSKY 1958). Thus lagging chromosomes can no longer reach the poles by the previous means, that is by activity of centromeres and half-spindles. Neocentric chromosome movement is observed in this hybrid, although not all lagging chromosomes exhibit this characteristic form and behavior. The attenuation of neocentric lagging chromosomes indicates that they are not passively moved to the poles by cytoplasmic currents (WALTERS 1952). Lagging chromosomes must reach the poles at late anaphase-early telophase by some means which does not involve the half-spindle, and in some instances at least by means other than passive movement by currents; perhaps by delayed formation and attachment of fibers from centromeres or neocentromeres to poles. The situation is not clear and not yet satisfactorily explained.

Lagging chromosome frequency appears to increase at prophase-II in the present hybrid, but this is because the definition of a "lagging chromosome" at that stage includes separate chromatids. The "increase" indicates that chromosomes excluded from the nucleus may divide and the chromatids separate between telophase-I and prophase-II.

The phragmoplast is generally considered to arise at anaphase as the half-spindles separate and finally to connect the poles of the former metaphase spindle. However, there are indications in this material that a phragmoplast does not always connect two spindle poles. Configurations have been described at late anaphase-telophase in which extra phragmoplasts connected one or two chromosomes, or even a fragment, and two large nuclei. The absence of these configurations at earlier anaphase indicates that they are very probably not tripolar. It was also observed that most lagging chromosomes do not have separate phragmoplasts, and that a lagging chromosome with its own phragmoplast is regularly located at the periphery of the equator; i.e., as far from the two large nuclei as possible.

It is known that centromeres and centrioles have characteristics in common (SCHRADER 1936, DARLINGTON 1937, POLLISTER and POLLISTER 1943). If centromeres of some lagging chromosomes were to assume characteristics of spindle organizers or spindle poles they could conceivably form separate phragmoplasts connected to the primary poles, and the peripheral position of these lagging chromosomes might then be attributable to repulsion between their centromeres and the poles. However, one phragmoplast configuration was found with only a

fragment at its apex. Because very little chromosome breakage occurs in this hybrid, it is much more probable that this is a one-break terminal fragment rather than a 2-break interstitial fragment which might contain a centromere. If an extra phragmoplast can be formed between an acentric fragment and a nucleus, obviously some explanation other than the above is necessary.

The phragmoplast increases in diameter at late anaphase. If it should simultaneously decrease in length (this occurs in *B. rubens* × *carinatus*, WALTERS 1958), and a peripheral lagging chromosome interfered with the shortening of fibers in its immediate vicinity, those longer fibers would have to bend as the major portion of the phragmoplast shortened. The point of the bend would then simulate a pole, with a single chromosome or chromatid at its apex, and the fibers extending from this point to the large nuclei would appear to be two separate phragmoplasts. The single fragment phragmoplasts (which were not as distinct as the others) would indicate that a very small portion of chromatin may have an influence on phragmoplast behavior. In the very rare example of a phragmoplast bent at some distance from lagging chromosomes, it is noted that this phragmoplast is also twice bent at the points of two lagging chromosomes; this distortion may have forced the bending of additional fibers.

The effects of subdividing the telophase-I chromosome complement and spindle material into several metaphase-II cells were previously described in the hybrid *B. rubens* × *carinatus* (WALTERS 1958). Now there is the opportunity to observe the behavior of the entire telophase-I chromosome complement and spindle substance present in a single metaphase-II cell. The metaphase-II configurations described above are: a single bipolar or tripolar spindle, two separate bipolar spindles, one bipolar and one tripolar, one 4-polar spindle, 3 separate bipolar spindles; two tripolar spindles were observed at anaphase-II.

It was suggested previously in *B. rubens* × *carinatus* that spindle organizers are instrumental in forming the spindle structure. Spindle organizers were visualized as compound structures, usually functionally single but capable of splitting into sub-units that also may be effective in spindle organization. A single telophase-I nucleus would contain one organizer; this presumably divides during prophase-II, and the two halves form the poles of the metaphase-II spindle (Fig. 54, A). Following this hypothesis, a single microsporocyte containing two telophase-I nuclei contains two organizers. If both divide as usual, two separate spindles are formed at metaphase-II (Fig. 54, A), and four telophase-II nuclei result. In some instances the two fail to divide during prophase-II, and one metaphase-II spindle is formed (Fig. 54, B) and two telophase-II nuclei. Division of only one organizer may result in formation of a single tripolar spindle (Fig. 54, C), and three telophase-II nuclei.

When three or four nuclei are present in one cell at prophase-II, it is possible that three or four organizers are also present, and these may form a 3-polar or 4-polar spindle (Fig. 54, D, E). If only two of the three organizers divide, the resulting 5 organizers may form one tripolar

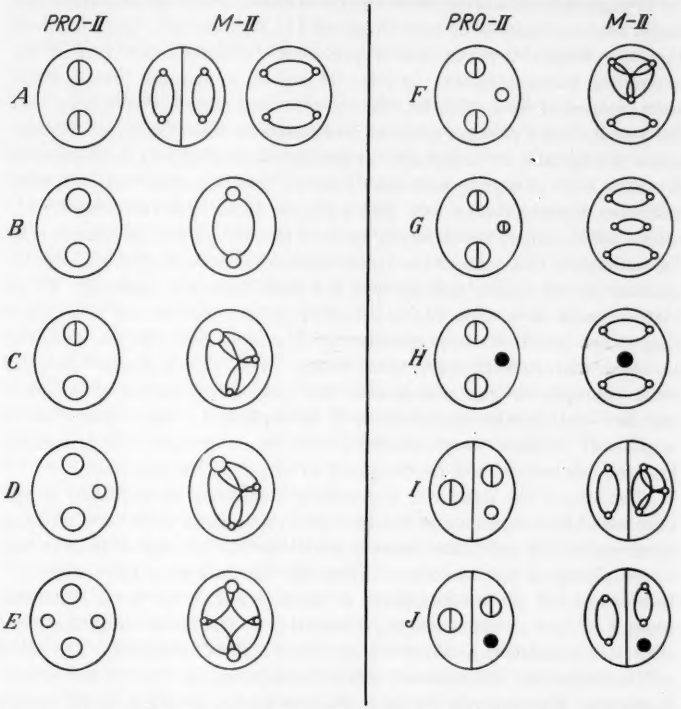


Fig. 54. Diagram showing various distributions and divisions of spindle organizers at prophase-II and the spindles resulting at metaphase-II

and one bipolar spindle (Fig. 54, F) and 5 nuclei at telophase-II. If all three are divided at prophase-II, three separate spindles may be formed (Fig. 54, G). It can be observed that one group of chromosomes may not be associated with any spindle; the organizer, if present, is inert. This microsporocyte may exhibit two separate spindles plus one clump of chromosomes at metaphase-II (Fig. 54, H), and 5 nuclei at telophase-II. There are many other possibilities.

The most common aberrant microsporocyte at prophase-II is a single cell containing three nuclei; and these are usually separated into only

two cells at metaphase-II. The most common aberrant microsporocyte at telophase-II is a dyad containing 5 nuclei. If the extra nucleus forms no spindle at metaphase-II, or if its organizer forms one pole of a tripolar spindle, 5 nuclei should result at telophase-II (Fig. 54, I, J).

Phragmoplasts can be observed to connect nuclei in multinucleate single microsporocytes at late anaphase-II (Figs. 50, 51). It is believed that the bulges between nuclei previously described are formed by expanding phragmoplasts. In Figs. 24 and 50 it appears that 5 nuclei were connected by 5 spindles. However, it has been shown above that the presence of a phragmoplast at late anaphase does not prove the presence of a spindle at metaphase. It has also been observed in endosperm divisions that phragmoplasts may form at late anaphase between nondaughter nuclei (BAJER and MOLÈ-BAJER 1954, GERASSIMOVA-NAVASHINA 1957). Therefore determination of the complexity of spindle configurations in this material is based on studies of metaphase-II microsporocytes. A 4-polar spindle was the most complex observed. In no instance were more than two separate spindles joined at one pole. Configurations in which three spindles are joined at one pole are observed in other materials (FANKHAUSER 1934). If in this material no more than two spindles can originate at one pole, the presence of separate tripolar and bipolar spindles in a metaphase-II microsporocyte is explained; if these were connected in a more complex configuration, at least one pole would be the point of origin of three spindles.

The size of the dyad cell is generally somewhat proportional to the number of chromosomes in it. In a microsporocyte with very unequal sized nuclei, the cell plate forms much closer to the smaller than to the larger group of chromosomes. Thus the chromosomes have some influence on cell plate formation. A third cell may be formed at telophase-I around a chromosome, chromatid, or fragment; thus the presence of a centromere is not necessary for cell plate formation. The third cell is formed at the equator, almost always at the side of the microsporocyte. Chromatin material located at the equator but in the center of the microsporocyte is generally cut through by the cell plate. Thus a certain orientation of chromatin material is necessary for cell plate formation, and it may be significant that the location of this chromatin is as far from the two nuclei as possible. These observations corroborate characteristics of cell division reported by FRANKEL (1949).

Extreme differences in rate of the two microsporocyte divisions are observed in this hybrid. The variation between anthers of a floret indicates that these differences are not caused by external environmental factors. The rate of cell plate formation shows some relation to the age of the anther; delayed cell division is most often found in older anthers, but there are some exceptions. The rate of cell division is often different

in different regions of the anther. It is possible that alterations in the rates of meiosis believed to occur in older anthers throw chromosome division and cell plate formation out of synchrony. SHIMAMURA and OTA (1956) have shown that polysaccharides are laid down across the phragmoplast along the line of cell plate formation; should their synthesis be delayed while chromosomes go through meiosis at a normal or accelerated rate, the observed delayed or incomplete cell divisions might occur.

It has been shown that cell division at telophase-II is somewhat delayed by the presence of lagging chromosomes. The delay is not attributable to a mechanical interference with cell plate formation, since these chromosomes are distributed throughout the cells. The effect of extra nuclei on cell plate formation is very much more pronounced. Not only is plate formation delayed at prophase-II and telophase-II but often it is incomplete, especially at prophase-II, and two nuclei are contained in one cell. It is also observed that when a third cell is formed around a chromatid or small nucleus these cell plates appear to form a little later than the primary plate. Phragmoplasts appear to form at an equal rate between all the nuclei of a multipolar configuration. Nevertheless it not only takes longer for three cell plates to form through three phragmoplasts than one to form through one phragmoplast, but the extra and usually smaller cell plates are the most delayed. Possibly a relationship between the distribution of polysaccharides and the unequal rate of cell division would be found.

Hybrids have previously been studied involving Santa Barbara *carinatus* and strains of *trinii* from Bishop and King City, California (WALTERS 1957). Neither of these exhibited the extreme range of meiotic stages or the delayed microsporocyte division found in the hybrid between Santa Barbara *carinatus* and Argentina *trinii*; in the former hybrids the tripolar spindle was rare. NAKAHARA and KOMOTO (1957) report that the range of meiotic stages is considerably increased and no predominant stage is present at the end of the growing season of *Tradescantia*. In the present hybrid, since only first flowering spikes were used, the effect is due to interaction of genes. The aberrations distinguishing this hybrid from its predecessors indicate that increasing genetic differences are related to increased geographical distances between the California and Argentina strains of *trinii*.

Summary

1. In the hybrid, *Bromus trinii* × *Bromus carinatus*, anthers were studied in which the proportion of microsporocytes in meiosis-I to those in meiosis-II ranged from 88:1 to 1:26. The different stages of meiosis occurred in various frequencies in individual anthers.

2. When anthers reach a certain stage of development the range of meiotic stages increases markedly. It is suggested that the rate of meiosis is altered, and variable among individual microsporocytes.

3. Multipolar spindles were observed at metaphase-I, metaphase-II, and multinucleate configurations at prophase-II and telophase-II. The separation of one nucleus into two was observed at late anaphase-I, prophase-II, late anaphase-II and telophase-II.

4. The multipolar spindles were generally tripolar. At prophase-II microsporocytes with 3 nuclei were most common, 4 nuclei were infrequent, 5 were rare. At telophase-II there were 2—7 nuclei per microsporocyte; 5 nuclei were most commonly found. Spindle poles were typically well separated; in multinucleate microsporocytes the nuclei were generally well separated.

5. The frequencies of multinucleate prophase-II microsporocytes varied from 0 to 36% in 41 anthers from 7 plants. The frequencies of telophase-II multinucleate microsporocytes ranged from 0 to 40% in the same anthers. There were no obvious differences between plants.

6. No correlation was found between frequencies of extra nuclei and lagging chromosomes. Therefore extra nuclei are believed to arise from subdivisions of existing nuclei; the formation of the latter is attributed to the previous establishment of spindle poles.

7. It is suggested that multipolar spindles and supernumerary nuclei arise from splitting or extra division of spindle organizers which may occur at various times throughout the meiotic cycles. It is further suggested that spindle organizers repel one another, most effectively during spindle formation, but to some extent also during other stages of meiosis.

8. Because multinucleate microsporocytes are generally found in older anthers, a relationship is suggested between the spindle irregularities that produce multipolar spindles and multinucleate microsporocytes and an altered, irregular rate of meiosis. Generally spindle aberrations are most often present at prophase-II when this stage is relatively infrequent, and at telophase-II when this stage is relatively abundant; however, the possible effect of differences in ages of anthers precludes the suggestion of a relationship between duration of meiotic stages and spindle irregularity. Frequencies of spindle aberrations are not necessarily constant in individual anthers, but may change during the course of the meiotic divisions.

9. Microsporocytes may divide normally into dyads at telophase-I and tetrads at telophase-II, or microsporocytes may remain single cells throughout meiosis. Extreme variations were found among individual anthers in the rate of microsporocyte division. Cell division tends to

occur normally in young anthers and to be delayed in older anthers. Normal and delayed division are described.

10. The presence of lagging chromosomes is related to a somewhat delayed microsporocyte division at prophase-II and telophase-II. The presence of extra nuclei is related to a very striking delay in division at prophase-II and telophase-II. Possible reasons for this delay are discussed.

11. A description is given of various spindle types observed in undivided microsporocytes at metaphase-II, and of the telophase-II configurations expected to result from these. It is suggested that the various types of spindles may originate by means of splitting of compound spindle organizers.

References

BAJER, A.: Studies on the spindle and chromosome movement. Acta Soc. Bot. Poloniae **21**, 99—111 (1951). — Cine-micrographic studies on chromosome movements in β-irradiated cells. Chromosoma (Berl.) **9**, 319—331 (1958).

—, and JADWIGA MOLÈ-BAJER: Endosperm — material for study on the physiology of cell division. Acta Soc. Bot. Poloniae **23**, 69—110 (1954). — Cine-micrographic studies on mitosis in endosperm. II. Chromosome, cytoplasmic and Brownian movements. Chromosoma (Berl.) **7**, 558—607 (1956).

BEADLE, G. W., and BARBARA MCCLINTOCK: A genic disturbance of meiosis in *Zea mays*. Science **68**, 433 (1928).

BÖÖK, J. A.: Cytological studies in *Triton*. Hereditas (Lund) **31**, 177—220 (1945)

CREW, F. A. E., and P. C. KOLLER: Genetical and cytological studies of the inter generic hybrid of *Cairina moschata* and *Anas platyrhyncha platyrhyncha*. Proc. Roy. Soc. Edinb. **56**, 210—241 (1936).

DARLINGTON, C. D.: Recent advances in cytology, 2nd edit. Philadelphia: P. Blakiston's Son & Co. 1937.

DUNCAN, R. E., and M. D. PERSIDSKY: The achromatic figure during mitosis in maize endosperm. Amer. J. Bot. **45**, 719—729 (1958).

EHRENBERG, L., and G. ÖSTERGREN: Experimental studies on nuclear and cell division. Bot. Not. **1942**, 203—206.

FRANKEL, O. H.: A self-propagating structural change in *Triticum*. I. Duplication and crossing-over. Heredity **3**, 163—194 (1949).

FANKHAUSER, G.: Cytological studies on egg fragments of the salamander *Triton*. V. Chromosome number and chromosome individuality in the cleavage mitosis of merogonic fragments. J. exp. Zool. **68**, 1—57 (1934).

GERASSIMOVA-NAVASHINA, HELEN: On some cytological principles underlying double fertilization. Phytomorphology **7**, 150—167 (1957).

HEITZ, E.: Beitrag zur Cytologie von *Melandrium*. Planta (Berl.) **1**, 241—259 (1926).

KNUDSEN, O.: Multiple spindles in the seminal epithelium of sterile bulls. Ark. Zool. **11**, 119 (1958).

KOLLER, P. C.: Spermatogenesis in *Drosophila pseudo-obscura* FROLOWA. II. The cytological basis of sterility in hybrid males of races A and B. Proc. roy. Soc. Edinb. **54**, 67—87 (1934).

MORINAGA, T., and E. FUKUSHIMA: Cytogenetical studies on *Oryza sativa* L. I. Studies on the haploid plant of *Oryza sativa*. Jap. J. Bot. **7**, 75—104 (1934).

NAKAHARA, K., and Y. KOMOTO: The effect of high temperature on the meiotic figures of *Tradescantia paludosa*. Cytologia (Tokyo) **22**, 1—11 (1957).

ÖSTERGREN, G.: The course of meiosis in a case of abnormally prolonged chromatid pairing. Ark. Zool. **11**, 130—131 (1958).

OKABE, S.: Über eine tetraploide Gartenrasse von *Psilotum nudum*, PALISOT DE BEAUVOIS (= *P. triquetrum* SW.) und die tripolige Kernteilung in ihren Sporenmutterzellen. Sci. Rep. Tohoku Imp. Univ., Ser. IV **4**, 373—380 (1929).

PEASE, D. C.: Hydrostatic pressure effects upon the spindle figure and chromosome movement. II. Experiments on the meiotic divisions of *Tradescantia* pollen mother cells. Biol. Bull. **91**, 145—169 (1946).

POLLISTER, A. W., and PRISCILLA F. POLLISTER: The relation between centriole and centromere in atypical spermatogenesis of viviparid snails. Ann. N.Y. Acad. Sci. **45**, 1—48 (1943).

SAKAMURA, T.: Experimentelle Studien über die Zell- und Kernteilung mit besonderer Rücksicht auf Form, Größe und Zahl der Chromosomen. J. Coll. Sci. Imp. Univ. Tokyo **39**, 1—221 (1920).

SCHRADER, F.: The kinetochore or spindlefiber locus in *Amphiuma tridactylum*. Biol. Bull. **70**, 484—498 (1936). — The spermatogenesis of the earwig *Anisolabis maritima* BON. with reference to the mechanism of chromosomal movement. J. Morph. **68**, 123—141 (1941).

SWANSON, C. P., and R. NELSON: Spindle abnormalities in *Mentha*. Bot. Gaz. **104**, 273—280 (1942).

THERMAN, EEVA, and S. TIMONEN: The prophase index and the occurrence of multipolar divisions in human cancer cells. Hereditas (Lund) **40**, 313—324 (1954).

WALSH, M. P., and J. B. MCMAHON: The cytological effects of OMPA. Rec. Genet. Soc. Amer. **22**, 109 (1953).

WALTERS, MARTA S.: Atypical chromosome movement in meiotic anaphase of *Bromus pitensis* × *B. marginatus*. Amer. J. Bot. **39**, 619—625 (1952). — Studies of spontaneous chromosome breakage in interspecific hybrids of *Bromus*. Univ. Calif. Publ. Bot. **28**, 335—447 (1957). — Aberrant chromosome movement and spindle formation in meiosis of *Bromus* hybrids: An interpretation of spindle organization. Amer. J. Bot. **45**, 271—289 (1958).

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BEITRÄGE ZUR ANALYSE DES BRASSICA-GENOMS*

Von

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Mit 10 Textabbildungen

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A. Einleitung

Die verschiedenen Artengruppen der Gattung *Brassica* sind durch die aneuploiden Chromosomenzahlen $n = 8$ (*B. nigra*), $n = 9$ (*B. oleracea*) und $n = 10$ (*B. campestris*) bzw. $n = 17$ (*B. carinata*), $n = 18$ (*B. juncea*) und $n = 19$ (*B. napus*) gekennzeichnet. Da die Ausgangsform dieser polyploiden Reihe unbekannt ist, versuchten frühere Autoren (Lit. vgl. bei YARNELL 1956), die Verwandtschaft der Arten innerhalb dieser Gattung mit folgenden indirekten Methoden aufzudecken: 1. sie verglichen die Morphologie der somatischen Chromosomen; 2. sie analysierten die Genomzusammensetzung von verschiedenen Arten durch eine cytologische Untersuchung interspezifischer Bastarde; 3. sie untersuchten das Paarungsverhalten der Chromosomen in haploiden Pflanzen; oder 4. sie beobachteten die Sekundärpaarung der Chromosomen in der Meiose der normalen diploiden Arten.

Besonders aufschlußreich waren Untersuchungen über die Chromosomenpaarung in Artbastarden. So kreuzte MORINAGA (1929) *B. napus*-Formen mit verschiedenen Varietäten von *B. campestris* und fand in der F_1 stets 10 Bivalente und 9 Univalente, während die reziproken Bastarde 9 Bivalente und 10 Univalente aufwiesen. Daraus zog er den Schluß, daß das Genom von *B. napus* aus einem *campestris*- und einem *oleracea*-Genom zusammengesetzt ist; dem entspricht, daß experimentell hergestellte Bastarde zwischen *B. campestris* und *B. oleracea* auch phänotypisch kaum von *B. napus* zu unterscheiden sind. In der gleichen Weise wurde die Amphidiploidie der *Brassica*-Arten mit 17 bzw. 18 Chromosomen nachgewiesen. Verwendet man die von MORINAGA (1934) eingeführten Bezeichnungen und nennt die Genome von *B. campestris* = a, *B. nigra* = b und *B. oleracea* = c, so bestehen die Genome der drei amphidiploiden Arten *B. carinata* aus b + c, *B. juncea* aus a + b und *B. napus* aus a + c. Soweit die bisher veröffentlichten cytologischen Untersuchungen erkennen lassen, sind beide Elterngenome in diesen Arten heute noch mehr oder weniger unverändert nebeneinander erhalten. Da aber bekannt ist, daß die Gestalt der einzelnen Chromosomen im Laufe der Evolution durch verschiedenartige Vorgänge abgewandelt werden kann, scheint der Zeitpunkt der natürlichen Bastardierung und Polyploidisierung in diesen Fällen phylogenetisch noch nicht weit zurückzuliegen.

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In Parallele zu solchen relativ einfachen und experimentell reproduzierbaren Artbildungsvorgängen deuten die cytologischen Untersuchungen an haploiden Pflanzen, sowie das Auftreten von Sekundärpaarung in der Meiose der *Brassica*-Arten mit den niedrigeren Chromosomenzahlen auf einen zweiten, komplexeren Vorgang hin, der zweifellos in der phylogenetischen Entwicklung schon sehr viel früher wirksam war und offensichtlich zur Entstehung der einfachen Genome a, b und c führte. Über den Ablauf dieser Ereignisse besteht jedoch bei den verschiedenen Autoren bis heute noch keine Einmütigkeit, da sich diese genomatischen Veränderungen nicht mehr im Kreuzungsexperiment wiederholen lassen. Man ist darum auf indirekte Methoden angewiesen, die in verschiedener Weise geeignet erscheinen, die Möglichkeiten der phylogenetischen Entwicklung dieser drei *Brassica*-Genome nachzuweisen.

Die meisten Untersuchungen gehen davon aus, daß in der Metaphase der 1. und 2. meiotischen Teilung häufig eine auffällige räumliche Zuordnung einiger Bivalente zueinander beobachtet wird. Das Zustandekommen einer solchen Sekundärpaarung wurde damit erklärt, daß nach Beendigung der Primärpaarung der homologen Chromosomen im Pachytän zwischen manchen Bivalenten noch gewisse Restpaarungskräfte wirksam werden können, die eine mehr oder weniger ausgeprägte Homologie auch dieser Chromosomen vermuten lassen. Da homologe Bivalente aber nur in polyploiden Individuen auftreten können, ergab sich die Vorstellung, daß auch die *Brassica*-Arten mit den niedrigeren Chromosomenzahlen Polyploide darstellen, die entweder durch Vermehrung einzelner Chromosomen eines hypothetischen „Urgenoms" oder durch Verdoppelung des ganzen Satzes und nachfolgenden Verlust einzelner Chromosomen entstanden sind und sekundär im gegenwärtig vorliegenden Zustand ein neues genetisches Gleichgewicht gefunden haben (CATCHESIDE 1934).

Diese cytologischen Hinweise wurden durch genetische Daten ergänzt, nach denen in diesen Arten eine ganze Anzahl von duplizierten Allelenpaaren vorkommt. PEASE (1926) wies z. B. zwei unabhängige Faktoren N_1 und N_2 nach, deren rezessive Allele bei *B. oleracea* Kopfbildung bedingen. Auch weitere Faktoren für die Kopfbildung, wie K_1 und K_2 (ALLGAYER 1928), sowie die Genpaare B_1 und B_2 für Verdickung der Sproßachse beim Kohlrabi (MALINOWSKI 1929), R_1 und R_2 für die Blattfarbe (KWAN 1934) oder W_1, W_2, W_3 und W_4 für die Kräuselung der Blätter (ALLGAYER) scheinen sekundär verdoppelt zu sein. Für die folgenden Erörterungen ist weiterhin von Interesse zu erwähnen, daß PEASE zwei von diesen Faktorenpaaren folgenden Koppelungsgruppen zuordnen konnte:

$$N_1\ P\ E\ W\ K_1$$
$$N_2\ T\ K_2$$

Das bedeutet aber, daß die entsprechenden duplizierten Chromosomen einander nicht mehr vollständig homolog sind.

Über die Chromosomenzahl des Urgenoms, also die Basiszahl der Gattung *Brassica* und die Art seiner Verwandtschaft mit den Chromosomensätzen a, b und c wurden die verschiedensten Ansichten geäußert. Diese Unsicherheit beruht darauf, daß die zugrunde liegenden cytologischen Untersuchungen nur in der Prometaphase oder der Metaphase der 1. oder 2. meiotischen Teilung durchgeführt wurden, in denen sich lediglich die räumliche Zuordnung von Chromosomen mehr oder weniger sicher erkennen läßt. Hingegen ist es in diesen Stadien nicht mehr möglich, die Homologie der sekundär assoziierten Chromosomen auch

anhand übereinstimmender Strukturmerkmale zu bestätigen. GOTTSCHALK (1954a) hat darum darauf hingewiesen, daß die „Sekundärpaarung als durchaus zuverlässige Testmethode für die Bearbeitung phylogenetischer Fragestellungen" nur dann herangezogen werden sollte, wenn man auch die zugehörigen früheren meiotischen Entwicklungsstadien — vornehmlich das Pachytän — mit in die Untersuchungen einbezieht. In diesem Stadium reichen die spezifischen, exakt erfaßbaren morphologischen Kennzeichen der Chromosomen aus, um feststellen zu können, inwieweit mit den beobachteten Assoziationen auch Homologien einhergehen. Im folgenden soll darum versucht werden, die Chromosomen der drei *Brassica*-Genome a, b und c im Pachytän individuell zu charakterisieren, um anhand ihrer Gestalt und ihres Paarungsverhaltens sicherere Hinweise auf die Entstehung und Zusammensetzung dieser Genome zu erhalten.

B. Material und Methode

Es wurden vergleichende Untersuchungen der Chromosomenmorphologie im Pachytän folgender Formen der Gattung *Brassica* durchgeführt:

B. campestris ssp. *oleifera* „Odenwälder Sprengelrübsen"
ssp. *pekinensis* (Herkunft Peking)
ssp. *narinosa* (Herkunft Debrecen)
ssp. *dichotoma* (Herkunft Chengchou)
ssp. *trilocularis* (Svalöf)

B. oleracea var. *acephala*, Markstammkohl „Littmanns Blattstammkohl"
Grünkohl „Lerchenzungen"
var. *capitata*, Rotkohl „Frührot"
var. *sabauda*, Wirsingkohl „Böckelmanns Westfalia"
B. nigra, Wildform (Herkunft Tripolis).

Ergänzend wurden Struktur und Paarungsverhalten der Chromosomen soweit wie möglich auch in der Diakinese dieser Arten sowie im Pachytän von experimentell hergestellten Artbastarden beobachtet. Letztere stammten aus Kreuzungen zwischen den Arten:

B. campestris ssp. *oleifera*, „Lembkes Winterrübsen"
B. oleracea var. *acephala*, Grünkohl „Lerchenzungen"
B. nigra, Wildform (Herkunft Elvas).

Die Antheren der jungen Blütenknospen wurden vollständig freipräpariert und in Alkohol-Eisessig = 3 : 1 fixiert. Nach 24 Std wurde in gesättigter (5%iger) Essigsäure-Karminlösung unter Zusatz von einer Spur Eisenacetat gefärbt. Eine längere Aufbewahrung der Antheren in der Fixierungslösung oder in 70%igem bzw. 96%igem Alkohol war auch bei einer niedrigen Temperatur von + 4° C stets von Nachteil; nach wenigen Wochen waren sie für Pachytänuntersuchungen unbrauchbar. Im Thermostaten bei max. 25° C waren die Pachytänchromosomen nach 8 Tagen ausreichend gefärbt. Die Quetschpräparate wurden im Phasenkontrast untersucht, alle individuell erkennbaren, unverzerrten Chromosomen mit einem Zeichenapparat etwa 3000fach vergrößert gezeichnet und ihre einzelnen Regionen nach der Zeichnung ausgemessen.

Ein Chromosom wurde dann als einwandfrei identifiziert angesehen, wenn seine charakteristischen Merkmale sowohl in mindestens 10 Pachytänkernen, als auch in wenigstens 10 verschiedenen Präparaten ausreichend übereinstimmten. Dabei wurde vor allem die Beschaffenheit des heterochromatischen Mittelsegments berücksichtigt, da dessen individuelle Gestaltung im Vergleich zu anderen Merkmalen, wie Chromosomenlänge und Schenkelquotient, in den einzelnen Präparaten einer sehr viel geringeren Variabilität unterliegt (GOTTSCHALK 1958). Wurden in einem Genom mehrere Chromosomen mit einem sehr ähnlichen oder gleich aussehenden Mittelsegment gefunden, so galt ihre Existenz erst dann als gesichert, wenn ihr gemeinsames Vorkommen in einem Kern mindestens fünfmal beobachtet worden war.

Für diese Feststellungen wurden nur solche Chromosomen verwendet, die in lockeren Kernen über ihre ganze Länge hin verfolgt werden konnten. Einzelne Bivalente, die aus Zellkernen mit stärker verknäueltem Chromatin zufällig herausgequetscht worden waren, wurden nicht ausgewertet.

In die Chromosomenschemata für die drei untersuchten Arten (Abb. 1) wurden als erste die Nukleolenchromosomen eingetragen und die übrigen nach der Menge ihres proximalen Heterochromatins geordnet. Die Längen der einzelnen Chromosomenabschnitte wurden jeweils als Mittelwerte aus mindestens 10 einzelnen Meßwerten dargestellt. Alle Abmessungen, deren Variabilitätskoeffizient (mittlerer Fehler in Prozent des Mittelwertes) die Zahl 10 überschreitet, sowie Strukturen, die nicht mit Sicherheit in jedem Chromosom desselben Typs zu finden waren, wurden mit einem „?" gekennzeichnet.

C. Ergebnisse

1. Die Struktur der Pachytänchromosomen

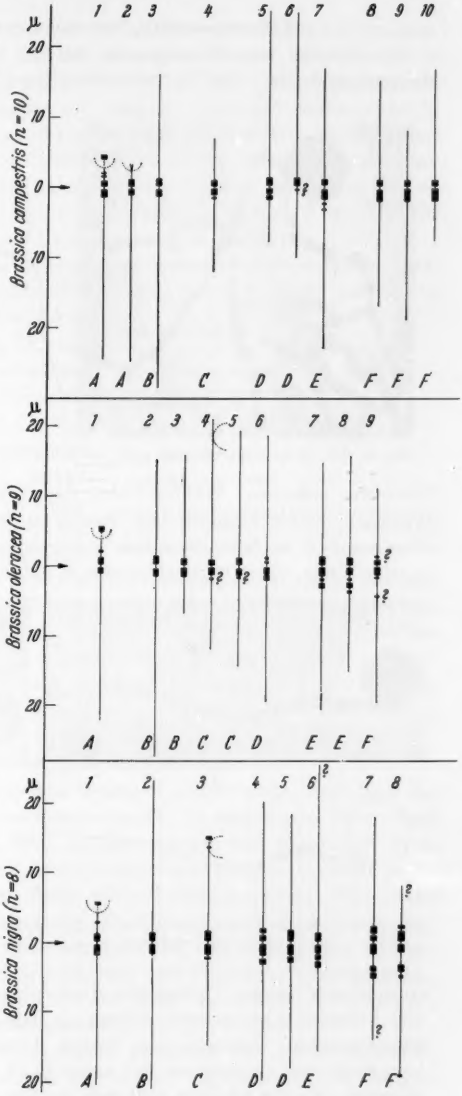
Über die Kennzeichen der Chromosomengestalt im Pachytän und ihre richtige Bewertung wurden in den vergangenen Jahren mehrere grundlegende Arbeiten veröffentlicht (LIMA DE FARIA 1952, GOTTSCHALK 1954b, LINNERT 1955, EBERLE 1956, OEHLKERS und EBERLE 1957, u.a.). Es erscheint darum nicht notwendig, an dieser Stelle auf die Technik und Problematik der „Pachytänanalyse" im einzelnen noch einmal näher einzugehen.

Wie in den frühesten meiotischen Prophasestadien sind die *Brassica*-Chromosomen auch noch im frühen Pachytän und dann schon wieder im Diplotän vollkommen verknäuelt und unmöglich zu identifizieren. Nur in einem sehr kurzen Stadium des späten Pachytäns ließen sich einzelne Chromosomen so weit isolieren, daß sie einwandfrei beobachtet werden konnten. Diese enge Begrenzung des analysierbaren Stadiums bot jedoch darin einen nicht zu unterschätzenden Vorteil, daß die Längendifferenzen, die durch die kontinuierliche Verkürzung der Chromosomen während der Prophase bedingt sind (vgl. EBERLE 1957, BAJER 1959), dadurch auf natürliche Weise eingeschränkt waren. In keinem Falle gelang es, alle Chromosomen eines Kernes gleichzeitig und vollständig zu erkennen. Lediglich sämtliche heterochromatischen Mittelsegmente waren in allen drei Arten mehrfach im gleichen Kern nebeneinander zu unterscheiden.

Die Chromosomen der drei untersuchten *Brassica*-Arten sind in distinkte, stark färbbare, heterochromatische Mittelstücke, die zu beiden Seiten des Centromers liegen, und daran anschließende euchromatische

Schenkel gegliedert. Während die distalen Chromosomenabschnitte locker schraubig aufgewunden sind, lassen die heterochromatischen Mittelsegmente keine innere Struktur erkennen. Sie sind bei einigen Chromosomen lediglich in sog. „Makrochromomeren" (im Sinne von GOTTSCHALK 1954b und LINNERT 1955) oder kleinere Heterochromatinblöcke aufgeteilt, bestehen in der Regel aber aus größeren heterochromatischen Segmenten. Im einzelnen lassen sich die Chromosomen durch die Zahl und Lage dieser heterochromatischen Abschnitte und durch die Länge ihrer Schenkel wie folgt charakterisieren und voneinander unterscheiden.

Die Lage des Centromers soll „median" genannt werden, wenn das Längenverhältnis des langen Chromosomenschenkels zum kurzen 1 bis 1,5, „submedian", wenn es 1,5 bis 2,5 und „subterminal", wenn es mehr als 2,5 beträgt. Mit „sehr kurz" seien die Pachytänchromosomen unter 20 μ Gesamtlänge, mit „kurz" solche zwischen 20 bis 25 μ, „mittel" 25 bis 30 μ, „lang" 30 bis 40 μ und „sehr lang" über 40 μ bezeichnet.


Brassica campestris (n = 10)
Brassica oleracea (n=9)
Brassica nigra (n=8)
µ
20
10
0
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20
1 2 3 4 5 6 7 8 9 10
A A B C D D E F F F
1 2 3 4 5 6 7 8 9
A B B C C D E E F
1 2 3 4 5 6 7 8
A B C D D E F F

Abb. 1. Schematische Darstellung der Pachytänchromosomen von drei *Brassica*-Arten

a) *B. campestris* („Odenwälder Sprengelrübsen")

Das Genom von *B. campestris* (Abb. 1) enthält zwei Nukleolenchromosomen (Nr. 1 und 2), die in der Regel beide dem einen großen

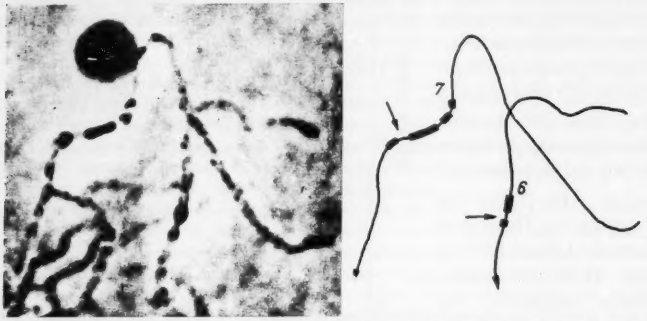


Abb. 2. Die Chromosomen *6* und *7* im Pachytän von *B. campestris*. Etwa 2470×

Nukleolus anhaften. Während aber das Nukleolenchromosom 1 einen typischen heterochromatischen Satelliten besitzt, fehlt dieser dem Chromosom 2, so daß dieses dem Nukleolus nur mit dem häufig ungepaarten Ende seines kurzen Schenkels aufliegt; daran ist es von dem

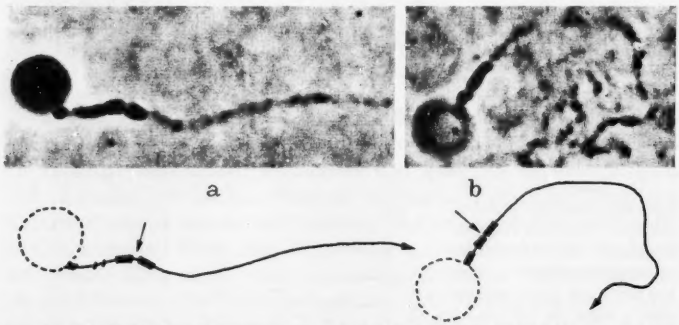


Abb. 3a u. b. Nukleolenchromosomen Nr. 1 im Pachytän von *B. campestris*. a ssp. *oleifera* „Odenwälder Sprengelrübsen". b ssp. *dichotoma*. Etwa 2470×

einzigen sehr langen Chromosom dieses Genoms, Nr. 3, und dem sehr viel kürzeren Chromosom 4 immer zu unterscheiden. Wie die zwei letztgenannten Chromosomen tragen beide Nukleolenchromosomen beiderseits des Centromers ein etwa gleich großes Heterochromatinsegment. Eine SAT-Zone ließ sich im Chromosom 2 nicht sicher erkennen. Chromosom 5 besitzt auf beiden Seiten seines Centromers einen

größeren bzw. zwei kleinere heterochromatische Abschnitte, Chromosom 7 einen auf der einen und drei auf der anderen Seite. Chromosom 6 ist vom Chromosom 5 daran zu unterscheiden, daß die beiden kleineren heterochromatischen Knöpfe im kürzeren Schenkel fehlen (Abb. 2). Die Chromosomen 8, 9 und 10 zeichnen sich durch vollständig identische Mittelsegmente aus; jedoch besitzt das lange Chromosom 9 ein medianes und die beiden anderen ein submedianes Centromer, wobei das kleinere Heterochromatinsegment im mittellangen Chromosom 9 im kürzeren und im kurzen Chromosom 10 im längeren Schenkel liegt.

Insgesamt besteht das *B. campestris*-Genom, wie wir es im Pachytän des „Odenwälder Sprengelrübsens" analysieren konnten, aus 1 sehr langen, 4 langen, 3 mittleren, 1 kurzen und 1 sehr kurzen Chromosom, von denen 4 Chromosomen ein subterminales und 2 ein mediales Centromer besitzen. Die Versuche, die Pachytänchromosomen auch in vier anderen *B. campestris*-Formen (s. S. 207) zu analysieren, ließen jedoch erkennen, daß vor allem die Chromosomenlänge, aber auch die Art der Heterochromatinverteilung innerhalb der Art variieren können. Allerdings reichen die mikroskopischen Befunde für die meisten Chromosomen noch nicht aus, um solche Differenzen bei den einzelnen Formen statistisch sichern zu können. Einige charakteristische Unterschiede im Feinbau des Nukleolenchromosoms 1 bei verschiedenen Subspecies von *B. campestris* zeigen Abb. 3 und das Schema Abb. 4.

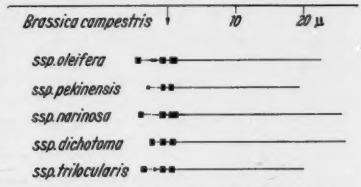


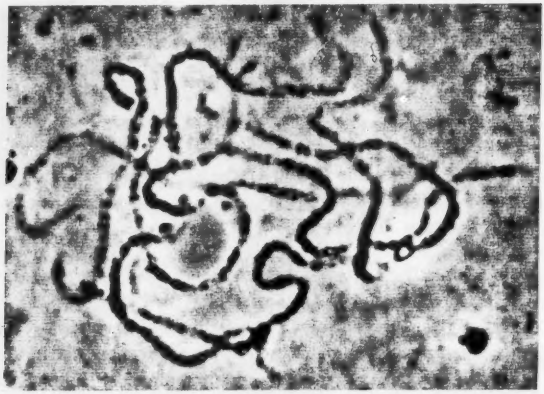
Abb. 4. Schematische Darstellung der Nukleolenchromosomen Nr. 1 im Pachytän verschiedener Subspecies von *B. campestris*

b) *B. oleracea* („Littmanns Blattstammkohl")

Das Schema des *B. oleracea*-Genoms (Abb. 1) zeigt, daß diese Art nur ein typisches Nukleolenchromosom (Nr. 1) besitzt, das durch einen heterochromatischen Satelliten auffällt. Häufig ließ sich jedoch beobachten, daß auch das Chromosom 4 mit einer bestimmten Stelle seines längeren euchromatischen Endes dem Nukleolus anhing. Hier, etwa ein Fünftel der Gesamtlänge des Schenkels vom Ende entfernt, liegt eine kurze achromatische Zone, mit der das Chromosom in allen 12 beobachteten Fällen, in denen es noch mit dem Nukleolus zusammenhing, an diesen befestigt war (Abb. 5). Der distal von dieser Stelle liegende Abschnitt ist also als euchromatischer Satellit anzusprechen.

Vergleichende Untersuchungen im Pachytän von drei Gemüsekohlformen (s. S. 207), die zwar bislang nur zu Teilanalysen ihrer Genome führten, zeigten jedoch, daß das Chromosom 4 in diesen Arten nicht als Satellitenchromosom ausgebildet ist. Es war deshalb in diesen

Abb. 5. Nukleolenchromosom 4 von *B. oleracea* mit euchromatischem Satelliten. Etwa 2470×



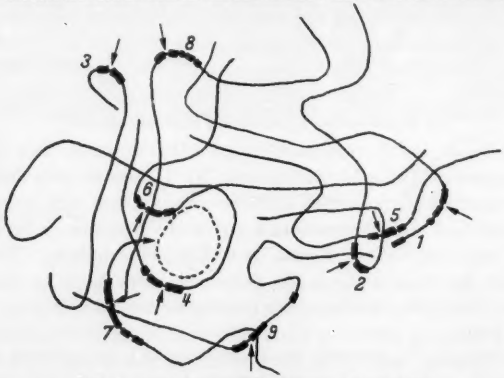


Abb. 6. Pachytän von *B. oleracea*. Etwa 2470×

B. oleracea-Varietäten nicht möglich, das 4. vom 5. Chromosom des Genoms zu unterscheiden. Entsprechend dieser strukturellen Übereinstimmung waren beide Chromosomen im Pachytän sehr häufig zu einem Multivalent vereinigt (vgl. Abschnitt 2, S. 216f.).

Die übrigen Chromosomen des *oleracea*-Genoms (vgl. Abb. 6) ähneln jeweils den im Schema (Abb. 1) darüberstehenden Typen von *B. campestris*. Man bemerkt in einigen Fällen eine Größenzunahme (Chromosomen 7 und 8) oder eine Vermehrung bzw. Aufteilung (Chromosomen 6 und 9) einzelner Elemente des heterochromatischen Mittelsegments. Sehr viel weniger korrespondieren die Längen der euchromatischen Schenkel, wenn auch das Chromosom 2 wieder (wie Chromosom 3 von *B. campestris*) das längste Chromosom des Genoms ist und die Chromosomen 7 und 9 auch in dieser Hinsicht den Chromosomen 7 und 8 von *B. campestris* ähneln.

Das Genom von *B. oleracea* setzt sich also aus 1 sehr langen, 5 langen, 2 mittleren und 1 kurzen Chromosom zusammen. Nur ein Centromer liegt subterminal (Nukleolenchromosom 1) und zwei submedian, die anderen Chromosomen sind mehr oder weniger symmetrisch.

c) *B. nigra* (Herkunft Tripolis)

Während man in den prämeiotischen Ruhekernen von *B. campestris* und *B. oleracea* jeweils nur zwei Nukleolenchromozentren zählt, sind bei *B. nigra* regelmäßig vier Chromozentren mit dem Nukleolus verbunden. Dementsprechend finden sich hier im Pachytänkern zwei Nukeolenchromosomen (vgl. Abb. 1). Das Nukleolenchromosom 1 ähnelt in seiner Morphologie dem Nukleolenchromosom 1 von *B. campestris* und *B. oleracea*. Das dem Nukleolenchromosom 3 von *B. nigra* entsprechende Chromosom 4 der beiden anderen Genome hingegen ist bei *B. campestris* nicht als Nukleolenchromosom ausgebildet und trägt bei *B. oleracea* nur einen euchromatischen Satelliten. Bei *B. nigra* jedoch besitzt Chromosom 3 einen deutlichen heterochromatischen Satelliten und proximal von der SAT-Zone ein zweites heterochromatisches Segment.

Abb. 7. Chromosom 8 im Pachytän von *B. nigra*. Etwa 2470 ×

Insgesamt ist die Heterochromatinmenge bei fast allen Chromosomen dieses Genoms größer als bei *B. campestris* oder *B. oleracea* (Abb. 7), und die Pachytänkerne sind noch stärker verknäuelt. Demzufolge konnte nicht von allen euchromatischen Schenkeln die Länge exakt bestimmt werden. Dennoch erlaubt die Struktur der heterochromatischen Centromerregion, jedes Chromosom individuell zu charakterisieren. Dabei ergaben sich dieselben Chromosomentypen, die auch in den Genomen von *B. campestris* und *B. oleracea* vorkommen. Trotz einiger Veränderungen durch Aufteilung oder Verschmelzung einzelner heterochromatischer Abschnitte ist die Ähnlichkeit der im Schema (Abb. 1) untereinanderstehenden Chromosomen unverkennbar.

Nach unseren Beobachtungen besteht das Genom von *B. nigra* aus 1 sehr langen, 4 langen und 3 mittellangen Chromosomen, von denen 5 symmetrisch gebaut sind.

d) Vergleich

Der Aufbau der drei Genome zeigt mancherlei Ähnlichkeiten. Diese betreffen nicht so sehr die Gesamtlänge der einzelnen Chromosomen oder deren Schenkel, als die Struktur ihrer heterochromatischen Mittelsegmente. Der Umfang dieser Zone nimmt in der Reihenfolge: *B. campestris* → *B. oleracea* → *B. nigra* etwas zu, beschränkt sich aber in allen drei Fällen auf relativ kurze proximale Abschnitte der Schenkel.

In allen drei Genomen sind vornehmlich nach der Gestaltung der Centromerregion 6 verschiedene Chromosomentypen zu unterscheiden. Die beiden Nukleolenchromosomen gehören entweder zum gleichen (B. *campestris*) oder zu verschiedenen Typen (*B. oleracea*, *B. nigra*).

In den einzelnen Genomen treten folglich einige Chromosomentypen mehrfach auf, und zwar kommen, wenn man sie wie in Abb. 1 mit Buchstaben bezeichnet, bei *B. campestris* die Chromosomen A und D zweimal und F dreimal, bei *B. nigra* D und F und bei *B. oleracea* B, C und E je zweimal vor. Demnach setzt sich das a-Genom aus den Chromosomen AA B C DD E FFF, das b-Genom aus A B C DD E FF und das c-Genom aus A BB CC D EE F zusammen.

2. Das Paarungsverhalten der Pachytänchromosomen

Die Ähnlichkeiten im Aufbau der drei untersuchten Genome und die Übereinstimmungen in der Struktur einiger Chromosomen deuten bereits auf eine enge Verwandtschaft dieser Arten hin. Ein Beweis für Homologien zwischen einzelnen Chromosomen oder Genomen kann jedoch nur in Untersuchungen über ihr Paarungsverhalten im Pachytän erbracht werden. Es wurde darum versucht, die Chromosomenpaarung in experimentell hergestellten F_1-Bastarden zwischen den drei Arten *B. campestris*, *B. oleracea* und *B. nigra* zu analysieren. Die verfügbaren amphihaploiden Bastarde mit den Genomen ab, ac und bc erwiesen sich aber für solche Beobachtungen als ungeeignet, da ihre Chromosomen sowohl im Pachytän als auch in der Diakinese stark verknäuelt waren. Es ließ sich darum lediglich feststellen, daß hier außer univalenten auch bivalente Chromosomenfäden vorkommen und daß insgesamt die Anzahl und Länge der gepaarten Chromosomenabschnitte im Bastard bc am niedrigsten und im Bastard ac am höchsten zu sein scheint.

In denselben Kreuzungsnachkommenschaften fanden sich aber in bestimmtem Umfange auch triploide Bastarde, deren Pachytänkerne

etwas weniger verklumpt waren. Abb. 8 zeigt eine Dreierkonfiguration aus einem aac-Bastard der aus einer F_1 von „Lembkes Winterrübsen" × Grünkohl stammte. Diese Figur, die in 14 verschiedenen Kernen auftrat, besteht aus drei langen Chromosomen mit je einem Heterochromatinblock beiderseits des Centromers. Offensichtlich handelt es sich bei den beiden eng gepaarten Strängen um die Chromosomen Nr. 3 aus den zwei a-Genomen und bei dem lockerer assoziierten um das Chromosom 2 aus dem Genom c. Die heterochromatischen Mittelsegmente dieser drei Chromosomen liegen in der Regel so dicht beieinander, daß sie meistens zu zwei dicken Klumpen verschmelzen. Ein

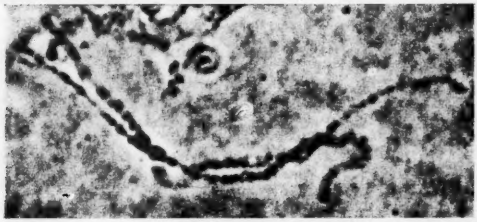


Abb. 8. Trivalent aus einem triploiden (aac) Bastard zwischen „Lembkes Winterrübsen" und Grünkohl, das sich aus zwei Chromosomen Nr. 3 der beiden a-Genome und einem Chromosom Nr. 2 des c-Genoms zusammensetzt. Etwa 2470 ×

Auswechseln der sich paarenden Partner an dieser Stelle konnte jedoch weder in dieser noch in anderen solchen Dreierfiguren eindeutig nachgewiesen werden. Auch scheint die Struktur der euchromatischen Schenkel des bivalenten Stranges nicht vollständig mit der des univalenten übereinzustimmen. Dennoch spricht die enge räumliche Zuordnung für unverkennbare Homologiebeziehungen zwischen diesen drei Chromosomen, die zwar demselben Typ B, jedoch verschiedenen Genomen zugehören. Weitere sekundäre Paarungsfiguren fanden wir in unseren aac-Bastarden sechsmal zwischen zwei *campestris*-Chromosomen Nr. 8 und einem *oleracea*-Univalent Nr. 9, sowie zweimal auch zwischen den Chromosomen 4 der beiden Elternarten. Im letzteren Falle war ein euchromatischer Schenkel des Univalents erheblich kürzer als der entsprechende des Bivalents.

In der Diakinese dieser Bastardpflanzen waren die Paarungsverhältnisse der Chromosomen infolge einer stärkeren Verklumpung der heterochromatischen Chromosomenabschnitte nicht mehr zu erkennen. Erst von der Metaphase an ließen sich die Univalente wieder deutlich von den Bivalenten und Trivalenten unterscheiden. Wie Tabelle 1 zeigt, sind nur in 12% der auswertbaren Kerne alle Chromosomen des c-Genoms univalent geblieben, hingegen in 86% ein oder zwei Chromosomen an Trivalentbildungen beteiligt. Ob diese Figuren durch Partner-

wechsel und Chiasmabildung entstehen oder ob sich die Bindung der drei Chromosomen bis in die Metaphase hinein nur durch eine bloße Verklebung der heterochromatischen Mittelsegmente erhält, war, wie erwähnt, nicht sicher zu entscheiden.

Die Dreierkonfigurationen zwischen zwei Chromosomen aus den beiden a- und einem aus dem c-Genom des Bastards können jedoch nur einen Teil der in Tabelle 1 aufgeführten Kombinationen erklären. Überall dort, wo die Summe der Bivalente und Trivalente höher als 10 ist, müssen sich zusätzlich zwei Chromosomen des c-Genoms zu einem Bivalent zusammengefügt haben. Die Mindesthäufigkeit einer solchen intragenomatischen Chromosomenpaarung ergibt sich aus Tabelle 1 zu 31%, wenn man die Häufigkeit, mit der diese Konfigurationen vorkommen, addiert.

Tabelle 1. *Konfigurationen in der Metaphase I des triploiden (aac) Bastards B. campestris oleifera × B. oleracea acephala*

Anzahl von Trivalenten (III), Bivalenten (II) und Univalenten (I) je Kern	Häufigkeit in Prozent (bei n = 435)
$10_{II} + 9_I$	12
$11_{II} + 7_I$	2
$1_{III} + 9_{II} + 8_I$	6
$1_{III} + 10_{II} + 6_I$	23
$2_{III} + 8_{II} + 7_I$	51
$2_{III} + 9_{II} + 5_I$	6

Solche Paarungsbeziehungen zwischen einzelnen Chromosomen desselben Genoms ließen sich auch im Pachytän normaler diploider Pflanzen der untersuchten *Brassica*-Arten nachweisen. Als Beispiel dafür stellt Abb. 9 eine Paarungsfigur zwischen den Bivalenten Nr. 4 und 5 von *B. oleracea* dar. Bei dieser stimmen je zwei der vier Schenkel morphologisch in allen Einzelheiten überein, sind also einander offensichtlich vollständig homolog. Eine Deutung dieser Konfiguration durch eine reziproke Translokation zwischen zwei nichthomologen Chromosomen ist somit ausgeschlossen, da die Kreuzfigur dann aus vier verschiedenen Schenkeln bestehen müßte. Zudem müßte sie in allen Pachytänkernen auftreten, sofern nicht die betroffenen Chromosomen ungepaart bleiben; das ist aber, wie Abb. 6 belegt, nicht der Fall. Ob diese Parungsfigur jedoch durch einen Partnerwechsel in der Centromerregion zustande kam, wie in der beigefügten Schemazeichnung angenommen wurde, oder lediglich auf einer Lateralassoziation der heterochromatischen Mittelsegmente beruht, ließ sich bei diesem Objekt mikroskopisch nicht eindeutig entscheiden. Es besteht jedoch kein Zweifel, daß es sich hier nicht nur um unspezifische Verklebungen des Heterochromatins handelt, da nur morphologisch ähnliche Bivalente vereinigt und stets die einander entsprechenden Heterochromatinblöcke miteinander in Paarung gefunden wurden. Für die Deutung als Partnerwechsel spricht, daß derartig eng konjugierte Bivalente sowohl in der Diakinese als auch noch in der Metaphase I der untersuchten *Brassica*-Arten nachgewiesen werden konnten, so z. B. beim Wirsingkohl in 27%, beim

Rotkohl in 36% und beim Grünkohl in 54% aller übersichtlichen Diakinesekerne.

In „Littmanns Blattstammkohl" allerdings zählt man in der Diakinese in 96% aller Fälle 9 einzelne Bivalente. Aus diesem Grunde wurde die morphologische Analyse des Pachytängenoms von *B. oleracea* an dieser Form durchgeführt. Die Homologie der Chromosomen 4 und 5 scheint hier also weniger ausgeprägt zu sein als in den drei erwähnten Gemüsekohlsorten. Dem entspricht, daß im Chromosom 4 der letzteren Varietäten niemals mit Sicherheit eine SAT-Zone nachgewiesen werden konnte, die dieses Chromosom in „Littmanns Blattstammkohl" charakteristisch vom Chromosom 5 unterscheidet.

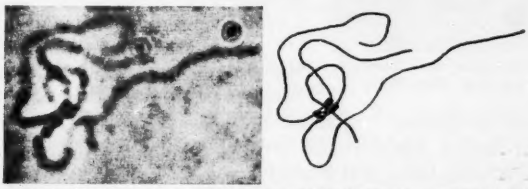


Abb. 9. Quadrivalent im Pachytän von *B. oleracea* aus den Chromosomen 4 und 5. Etwa 2470 ×

Sekundärpaarung fand sich in „Littmanns Blattstammkohl" zweimal auch zwischen den Chromosomen 2 und 3 und einmal zwischen den Chromosomen 7 und 8. Sie ließ sich ebenfalls im Pachytän der beiden anderen untersuchten *Brassica*-Arten nachweisen. In allen beobachteten Fällen waren ausschließlich die heterochromatischen Mittelsegmente gepaart bzw. durch einen Partnerwechsel verbunden.

Bei *B. campestris* („Odenwälder Sprengelrübsen") wurde zweimal eine Paarungsfigur zwischen den Chromosomen 5 und 6 festgestellt, in der die beiden kurzen Schenkel proximal etwa zur Hälfte ungepaarte, also offensichtlich inhomologe Chromosomensegmente enthielten. Die Strukturveränderung, die demnach in diesen Abschnitten stattfand, änderte auch das heterochromatische Mittelsegment des Chromosoms 6 und damit ein wichtiges Charakteristikum dieses Chromosoms. Erst der Nachweis dieser Paarungsbeziehungen ermöglichte darum, das Chromosom seinem richtigen Strukturtyp zuzuordnen.

Bei *B. nigra* machte die stärkere Verklumpungstendenz der heterochromatischen Mittelsegmente eine genaue Analyse der auch hier auftretenden Sekundärpaarungen nahezu unmöglich. Aus demselben Grunde war eine quantitative Analyse des Partnerwechsels im Pachytän bei keiner der drei Arten zu erreichen.

Auch in der Diakinese hängen die Chromosomen von *B. campestris* und vor allem von *B. nigra* regelmäßig in Gruppen aneinander. Es

gelang darum nur bei *B. oleracea*, aus der Anzahl der Multivalentfiguren in der Diakinese auf die Häufigkeit von Sekundärpaarung im Pachytän zurückzuschließen. Beim Grünkohl trat, wie erwähnt, in 54% der 438 analysierten Zellen eine Viererkonfiguration auf, die zuweilen auch noch in der späten Metaphase wiedergefunden werden konnte. Mit einer Häufigkeit von 2% kamen Paarungsfiguren zwischen drei Bivalenten (den Chromosomen 1, 2 und 3?), die stets in unmittelbarer Nähe des Nukleolus lag (Abb. 10), und in 0,7% zwei Vierergruppen in derselben Zelle (Chromosomen 2 und 3 bzw. 4 und 5?) vor. Im letzteren Falle enthielt ein Multivalent stets ein deutlich kleineres Bivalent (wohl das Chromosom 3).

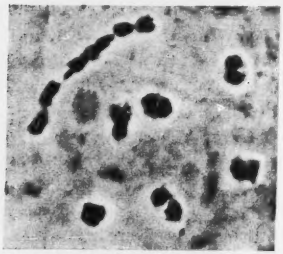


Abb. 10. Diakinese von *B. oleracea* mit Hexavalent. Etwa 2040 ×

In der Metaphase I beobachteten wir in allen drei untersuchten Arten in einigen Zellen (unter je etwa 1000 analysierten Kernen in 3,9% bei *B. campestris*, in 11,6% bei *B. oleracea* und in 5,9% bei *B. nigra*), daß einzelne Bivalente zu zweit oder selten auch einmal zu dritt deutlich

Tabelle 2. *Häufigkeit von Sekundärpaarung in der Metaphase I der Pollenmutterzellen von drei Brassica-Arten*

B. campestris		*B. oleracea*		*B. nigra*	
Bivalentkombinationen[1]	Gefundene Anzahlen	Bivalentkombinationen	Gefundene Anzahlen	Bivalentkombinationen	Gefundene Anzahlen
10 (1)	1092	9 (1)	860	8 (1)	844
1 (2) + 8 (1)	9	1 (2) + 7 (1)	44	1 (2) + 6 (1)	32
2 (2) + 6 (1)	19	2 (2) + 5 (1)	52	2 (2) + 4 (1)	21
3 (2) + 4 (1)	11	3 (2) + 3 (1)	17		
4 (2) + 2 (1)	2				
1 (3) + 1 (2) + 5 (1)	1				
1 (3) + 2 (2) + 3 (1)	2				
	1136		973		897

[1] Anzahl der Bivalente je Zelle, die einzeln liegen (1) bzw. zu zweit (2) oder zu dritt (3) sekundär gepaart sind.

zusammengerückt waren. Manche lagen in enger Berührung nebeneinander, andere weiter voneinander entfernt, aber doch unverkennbar einander zugeordnet. Im einzelnen zeigt Tabelle 2 die Häufigkeit einer derartigen sekundären Paarungserscheinung in der Metaphase I. Gelegentlich wurden ähnliche Assoziationen auch in der Metaphase II beobachtet.

Im Pachytän der drei untersuchten *Brassica*-Arten werden also zwischen einzelnen Chromosomen mit ähnlicher oder übereinstimmender Struktur Paarungsaffinitäten wirksam, die in verschiedener Häufigkeit zu Sekundärpaarung führen. Bei *B. oleracea* waren am häufigsten die Chromosomen 4 und 5 miteinander verbunden, d.h. die beiden Chromosomen des Genoms, die einander auch morphologisch am meisten gleichen. Es konnten jedoch anhand von Paarungsfiguren eines triploiden Bastards Homologien auch noch zwischen den Chromosomen aus verschiedenen Genomen, die aber zum gleichen Strukturtyp gehören, aufgezeigt werden.

D. Diskussion

1. Versuche einer Genomanalyse auf Grund der Länge und Symmetrie der Chromosomen

Catcheside (1934), Alam (1936) und Richharia (1937a, b) versuchten, die Chromosomen in den Genomen mehrerer *Brassica*-Arten in der Mitose morphologisch zu identifizieren. Richharia (1937b) z.B. maß die Gesamtlänge und Symmetrieverhältnisse der mitotischen Chromosomen von mehreren 10-chromosomigen Arten und stellte, entsprechend unseren Befunden im Pachytän, fest, daß in diesen a-Genomen 6 verschiedene Chromosomentypen vorkommen. Vergleicht man in Tabelle 3, wie dieser Autor die 10 Chromosomen des haploiden Satzes beschreibt, so stimmt seine Charakteristik des Genoms auch im einzelnen mit der unseren recht gut überein. Richharia stellte anhand

Tabelle 3. *Morphologischer Vergleich der Chromosomen von B. campestris in der mitotischen Metaphase* (Richharia 1937b) *und im Pachytän*

Mitose		Meiose	
Chromosomentyp (nach Richharia)	Chromosomengestalt	Chromosomentyp	Chromosomengestalt
A	sehr lang, submedian	B	sehr lang, submedian
B	lang, fast median	E	lang, median
C	lang, subterminal	A	mittel, subterminal
D	lang, submedian	D	lang, subterminal
D	lang, submedian	D	lang, subterminal
E	mittel, subterminal	A	mittel, subterminal
E	mittel, submedian	F	lang, median
F	kurz, median	F	mittel, submedian
F	kurz, submedian	F	kurz, submedian
F	kurz, submedian	C	sehr kurz, submedian

seiner Ergebnisse für das $2n$-Genom von *B. campestris* jedoch die Formel AA BB CC DDDD EEEE FFFFFF auf, während wir die Pachytänchromosomen mit AA B C DD E FFF bezeichneten. In beiden Fällen wird also angenommen, daß zwei Chromosomen doppelt und eines

dreifach vorhanden ist. Nach unseren Befunden ist aber die Bezeichnung der Chromosomen ausschließlich nach ihrer Länge ungeeignet, wenn eine Antwort auf die Frage nach ihrer Homologie gesucht wird. Als Beispiel sei auf die Beobachtung hingewiesen, die RICHHARIA selber in der zitierten Arbeit beschreibt, daß sich in der Metaphase I nicht selten auch Bivalente von verschiedener Größe sekundär paaren können (vgl. S. 218). Dieser Befund läßt sich aber mit seiner eigenen Genomformel nicht erklären; denn wenn z. B. die drei kurzen Chromosomen durch Vermehrung ein und desselben Ausgangschromosoms entstanden sein sollen und alle anderen Chromosomen des Genoms zu einem anderen Typ gehören, ist eine solche Sekundärpaarung unverständlich. Nimmt man jedoch an, daß die beobachteten Paarungsbilder durch die Chromosomen 8 und 10 (nach unserer Numerierung), also das lange und das kurze Chromosom desselben Typs F zustande kamen, so läßt sich gerade dieser Befund einer Sekundärpaarung als ein weiteres Anzeichen für eine partielle Homologie dieser ungleich großen Bivalente werten.

RICHHARIA (1937b) untersuchte die somatischen Chromosomen in drei verschiedenen Subspecies des Artkomplexes *B. campestris* und beschrieb das Genom der ssp. *chinensis* mit der Formel AA BB CC DDDD EEEE FFFFFF, der ssp. *chinensis* mit AA BB CC DDD'D' EEEE FFFFFF und der ssp. *rapa* mit AA BB CC DDD'D' EEEEEE FFFF. Auch nach unseren Befunden im Pachytän besteht kein Zweifel, daß die Genome der verschiedenen Subspecies dieser Art morphologisch sicherlich nicht in allen Chromosomen vollständig übereinstimmen. Allerdings müßten, nach den vorstehend diskutierten Erfahrungen zu urteilen, die Unterschiede im einzelnen wohl erst noch im Pachytänstadium näher analysiert werden, ehe ähnliche Formeln als gesichert gelten dürfen.

Dieselben Erörterungen, wie sie für das Genom von *B. campestris* angestellt wurden, ließen sich auch für andere *Brassica*-Arten mit demselben Ergebnis wiederholen. So fand SIKKA (1940) in Mitosen von *B. nigra*, wie wir im Pachytän, 1 sehr langes, 4 lange und 3 mittellange Chromosomen. Auch seine Klassifizierung des mitotischen Chromosomenansatzes von *B. oleracea* mit 1 sehr langen, 4 langen, 3 mittleren und 1 kurzen Chromosom entspricht genau der unseren, wenn wir das Chromosom 8 mit 30,9 μ Pachytänlänge noch als mittellang ansprechen. Es sei erwähnt, daß SIKKA hier außer einem subterminalen ein langes Chromosom mit submedianer Insertion als Nukleolenchromosom beschreibt, also offensichtlich auch in der von ihm verwendeten Varietät unser Chromosom 4 als SAT-Chromosom ausgebildet war. Über das Problem der Homologie einzelner Chromosomen können aber alle derartigen Untersuchungen in der Mitose, wie erwähnt, keine sicheren Aufschlüsse geben.

2. Versuche einer Genomanalyse auf Grund des Paarungsverhaltens der Chromosomen

Die Paarung der Chromosomen in der Meiose ist zweifellos ein sehr viel sicherer Weg, Homologien zu erkennen. Verschiedene Autoren haben darum versucht, das Problem der sekundär balancierten Polyploidie der drei auch von uns untersuchten *Brassica*-Arten auf diesem Wege anzugehen. Sie machten dabei von zwei Möglichkeiten Gebrauch und beobachteten entweder die Chromosomenpaarung in spontan auftretenden Haploiden bzw. in Bastarden, in denen einzelne Genome haploid vorlagen (a), oder untersuchten die Sekundärpaarung von Bivalenten in der Metaphase der 1. oder 2. meiotischen Teilung (b).

a) Die Chromosomenpaarung innerhalb haploider Genome

Die Methode, das Paarungsverhalten von Chromosomen zu untersuchen, die nur in haploider Anzahl in die Meiose eintreten, ist deshalb besonders aufschlußreich, weil sich gezeigt hat, daß sich die Chromosomen im diploiden Kern auch dann ausschließlich zu zweit paaren können, wenn einzelne Chromosomenstücke oder selbst ganze Chromosomen verdoppelt, also vierfach vorhanden sind. Tatsächlich hat das Studium der Meiose von Haploiden vielfach gezeigt, daß hier, wo die übliche Homologenpaarung ausgeschaltet ist, Paarungsmöglichkeiten verwirklicht werden, die infolge von Verdoppelungen ganzer Genome, einzelner Chromosomen oder von Chromosomenstücken im haploiden Satz vorhanden sind.

In der Gattung *Brassica* wurde eine solche Untersuchung von Ramanujam (1941) an haploidem *B. campestris* ssp. *toria* durchgeführt. Dabei fanden sich in der Metaphase I zwar in der Regel 10 Univalente, aber auch einige Pollenmutterzellen mit 1 bis 3 Bivalenten. In Haploiden von *B. oleracea* zählte Thompson (1956) in 54 Metaphasezellen 12 Kerne mit einem und 4 mit zwei Bivalenten. Haploide Pflanzen von *B. nigra* wurden unseres Wissens noch nicht cytologisch untersucht.

Es liegen jedoch weitere Beobachtungen an amphihaploiden *Brassica*-Formen (Olsson und Hagberg 1955) vor, d.h. an experimentell hergestellten oder natürlichen haploiden Individuen der amphidiploiden Arten. Mizushima (1952) stellte aus Ergebnissen älterer Arbeiten zusammen, daß in F_1-Bastarden mit den Genomen ab maximal 3, bc maximal 4 und ac maximal 8 Bivalente vorkommen können. Entsprechendes beschrieben Olsson und Hagberg in der Metaphase I von haploiden Rapspflanzen. Diese Autoren führten die Paarung zu 2 bis 8 oder 9 z.T. ausgesprochen heteromorphen Bivalenten ausschließlich auf eine partielle Homologie von je einem Chromosom des *campestris*- mit einem *oleracea*-Genoms zurück. Es ist jedoch schon nach den vorstehend erwähnten Ergebnissen aus Untersuchungen an haploiden Pflanzen der beiden Ausgangsformen (*B. campestris* und *B. oleracea*)

nicht auszuschließen, daß ein Teil der Bivalente in diesen haploiden Rapspflanzen auch durch Paarungen innerhalb eines Satzes zustande gekommen sein kann. Für diese Möglichkeit sprechen auch die eigenen Ergebnisse über die Chromosomenpaarung in semisynthetischen Rapspflanzen mit der Genomzusammensetzung aac, in denen zweifellos wenigstens zwei Chromosomen des c-Genoms zu einer Bivalentbildung fähig sind. Ähnliche Beobachtungen teilte kürzlich auch SHIBATA (1956) mit; nach seinen Befunden sind die Paarungsfiguren in der Metaphase eines Bastards zwischen *B. pekinensis* und *B. napus* nur so zu deuten, daß man 5 bis 7 Möglichkeiten einer Allosyndese zwischen Chromosomen der Genome a und c und mindestens eine autosyndetische Paarung inneshalb des Genoms c annimmt. Die Anzahl der Beispiele ließe sich anhand der älteren Literatur, die in den umfangreichen Sammelreferaten von YARNELL (1956) und MIZUSHIMA (1952) zusammengetragen wurde, beliebig vermehren. Wenn auch in haploiden Genomen eine Paarung inhomologer Chromosomensegmente nicht immer vollständig ausgeschlossen werden kann (vgl. RIEGER 1957), so ist doch auf Grund der Regelmäßigkeit, mit der solche Paarungserscheinungen von den verschiedensten Autoren beobachtet wurden, als wahrscheinlich anzunehmen, daß sie auf eine wenigstens partielle Homologie der beteiligten Chromosomen zurückgehen. Allenfalls erscheint die Möglichkeit einer Chromosomenpaarung zwischen den drei *Brassica*-Genomen a, b und c nach allen Befunden ebenso sicher wie die Konjugation einzelner Chromosomen innerhalb dieser Genome.

Abschließend seien in diesem Zusammenhang einige Beobachtungen erwähnt, deren genauere Analyse dazu beitragen könnte zu erkennen, wie sich die einzelnen Genome im Laufe der Evolution in verschiedener Weise strukturell verändert haben. RAMANUJAM und SRINIVASACHAR (1943) untersuchten die Chromosomenpaarung in einer haploiden Pflanze von *B. juncea*. Sie fanden zwar eine geringe Anzahl von Bivalenten, jedoch sehr viel weniger, als in einem experimentell hergestellten F_1-Bastard zwischen *B. campestris* und *B. nigra*. Daraus folgerten sie, daß die Reduktion der Chromosomenpaarung in dieser Haploiden möglicherweise dadurch zustande gekommen ist, daß die gegenseitige Homologie der beiden Elterngenome von *B. juncea* seit der Entstehung des amphidiploiden Zustandes abgenommen hat. Demgegenüber stellten OLSSON und HAGBERG in haploiden Rapspflanzen im Vergleich zu einem F_1-Bastard zwischen *B. campestris* und *B. oleracea* (U 1935) keine Abnahme der Paarungsaffinität fest und nahmen darum an, daß *B. napus* phylogenetisch jünger als *B. juncea* ist.

b) Die Sekundärpaarung

Diese Befunde über vielfache Homologiebeziehungen innerhalb sowie zwischen den einzelnen *Brassica*-Genomen, wie sie sich aus den Paarungsverhältnissen in haploiden Genomen ergeben, wurden verschiedentlich dadurch bestätigt, daß in der Metaphase I und II eine „Sekundärpaarung" einzelner Bivalente auftrat. Als erster beobachtete

CATCHESIDE (1934) bei einigen *Brassica*-Formen, daß die Gemini in der Metaphase nicht zufällig verstreut, sondern in lockeren Gruppen zu zweit oder zu dritt nebeneinander liegen können. DARLINGTON (1927) und LAWRENCE (1929, 1931), die solche „sekundären Assoziationen" als letzte Auswirkung einer ehemals vollständigen Homologie der beteiligten Bivalente auffaßten, beschrieben als besondere Kennzeichen dieser Erscheinung, daß sie erst in der Prometaphase I oder II auftritt und sich die Chromosomen zwar paarweise oder zu mehreren zusammenlegen, aber dabei nicht berühren (Lit. vgl. bei GOTTSCHALK 1955).

Die meisten Autoren, die bei den verschiedenen *Brassica*-Arten sekundäre Paarungsfiguren beschreiben (CATCHESIDE 1934, 1937; ALAM 1936; RICHHARIA 1937a, b; HAGA 1938a, b), beobachteten in einem Kern niemals weniger als 6 Chromosomengruppen. Die maximale Anzahl sekundär assoziierter Bivalente betrug also bei *B. campestris* 4, *B. oleracea* 3 und *B. nigra* 2. Die vorliegenden eigenen Befunde (Tabelle 2) stimmen mit diesen Ergebnissen überein. Die Zahl 6 ist demnach als Basiszahl der drei *Brassica*-Genome durchaus wahrscheinlich. SIKKA (1940) jedoch fand bei *B. nigra* auch drei Bivalentpaare sekundär gepaart und nimmt darum 5 als Grundzahl der Gattung an. Diese Diskrepanz läßt sich durch Auszählung von Sekundärpaarungsfiguren allein nicht beseitigen, da es in einzelnen Fällen unmöglich ist, unspezifische Chromosomenverklebungen und -zusammenlagerungen von einer wirklichen Sekundärpaarung zu unterscheiden.

In unseren Untersuchungen stellten wir in der Metaphase I Sekundärpaarung höchstens in etwa 10% aller analysierten Kerne fest, während z.B. CATCHESIDE (1937) dieselbe Erscheinung bei *B. oleracea* in 90% aller Zellen beobachtete. Zur Erklärung dieser Differenz könnte man sowohl genetische als auch Umweltfaktoren heranziehen. Mit Sicherheit ist als wirksamer Faktor aber nur anzuführen, daß die hier verwendete Quetschmethode (gegenüber Ausstrich und Paraffintechnik) für eine Erhaltung von Lagebeziehungen im Kernraum zweifellos wenig geeignet ist.

Andererseits muß betont werden, daß sich in unseren Präparaten, von der klassischen Definition des Begriffes abweichend, viele der assoziierten Bivalente in der Metaphase I deutlich berührten und entsprechende Kombinationen bereits in der Diakinese, ja schon im Pachytän nachgewiesen werden konnten. Diese Gruppierungen lassen sich wahrscheinlich auf Partnerwechsel zurückführen. Demgegenüber ist die Entstehung der übrigen, auf „Distanz konjugierten" Chromosomengruppen schwieriger zu erkennen. GOTTSCHALK (1954a, b) vertritt die Ansicht, daß beide Möglichkeiten der räumlichen Zuordnung einzelner Bivalente ein Ausdruck desselben Phänomens sind (obwohl er selber in den von ihm untersuchten Solanaceen eine Sekundärpaarung nur

im Pachytän, aber nicht mehr in der Metaphase nachweisen konnte; vgl. GRÖBER 1958). Je nach dem Grad der Homologie zweier Gemini, der sich im Pachytän z.T. schon an der Chromosomenstruktur ablesen läßt, ziehen sich die einander entsprechenden Abschnitte der Bivalente mehr oder weniger stark an, so daß alle Übergänge von einer lockeren Parallellagerung bis zu einer engen Berührung ganzer Bereiche mit der Möglichkeit zu Partnerwechsel vorkommen können. Daß wir im Pachytän unserer *Brassica*-Arten keine eindeutigen Fälle einer „Distanzkonjugation" einzelner Bivalente fanden, mag z.T. auf die relativ starke Verknäuelung der Kerne zurückgehen. Zudem ist man geneigt, Präparate, in denen einzelne oder mehrere Chromosomen dicht nebeneinanderliegen oder gar aneinanderhängen, wie es in der Diakinese von *B. campestris* und vor allem von *B. nigra* meistens der Fall ist, als schlecht fixiert und artefiziell verändert anzusehen, was erklären könnte, daß auch in diesem Stadium keine derartigen Paarungsbilder auffielen. Die Vorstellung von GOTTSCHALK (1954b), daß die Sekundärpaarung homologer Bivalente im Pachytän und die „sekundäre Assoziation" in der Metaphase die gleiche Erscheinung in verschiedenen meiotischen Stadien darstellt, scheint sich damit, was die von uns untersuchten *Brassica*-Arten angeht, zu bestätigen. Allenfalls konnten wir Sekundärpaarung in der vorliegenden Untersuchung bei dem gleichen Objekt sowohl im Pachytän als auch in der Metaphase nachweisen. Ein entsprechendes Verhalten war bisher nur in der Gattung *Carex* (HEILBORN 1936) und bei *Digitalis purpurea* (LINNERT 1949) bekannt.

3. Versuch einer Genomanalyse auf Grund der Chromosomenstruktur im Pachytän

Alle Befunde über die Chromosomenpaarung in haploiden sowie über die Sekundärpaarung in diploiden Genomen der Arten *B. campestris*, *B. oleracea* und *B. nigra* zeigen, daß sowohl zwischen als auch innerhalb von diesen Genomen zahlreiche Homologien bestehen. Aber erst die genaue Analyse der Chromosomenstruktur im Pachytän erlaubt es, die einzelnen Chromosomen zu erkennen, die einander jeweils mehr oder weniger homolog sind. Unsere Untersuchungen in diesem Stadium ergaben, daß die drei *Brassica*-Genome a, b und c aus 6 morphologisch verschiedenen Chromosomentypen zusammengesetzt sind, von denen — in einzelnen Genomen unterschiedlich — einige mehrfach vorkommen. Um das zu veranschaulichen, wurde für jedes Genom eine „Genomformel" aufgestellt. Die Formeln, die lediglich anhand der Strukturtypen der Pachytänchromosomen ermittelt wurden, entsprechen grundsätzlich den Genomformeln, die einige frühere Autoren (CATCHESIDE 1937, RICHHARIA 1937a, b, HAGA 1938a, b) anhand der Chromosomen-

morphologie in der Mitose oder der Befunde über die Sekundärpaarung ableiteten. Lediglich die Zuordnung der Buchstaben zu den einzelnen Chromosomentypen differiert aus Gründen, die bereits auf S. 219f. erörtert wurden.

Darüber hinaus bestätigen unsere Strukturschemata der Pachytänchromosomen erneut, daß morphologische Übereinstimmung und Paarungsaffinität der Chromosomen eng miteinander verknüpft sind. Wenn man z. B. die Ähnlichkeiten der einzelnen Chromosomen in Abb. 1 vergleicht, so fällt es nicht schwer zu verstehen, daß sich bei *B. oleracea* maximal drei Chromosomenpaare in Sekundärassoziation fanden und daß ein Partnerwechsel zwischen den Chromosomen 4 und 5 offensichtlich am häufigsten und zwischen den Chromosomen 2 und 3 bzw. 7 und 8 seltener vorkommt. Das bedeutet andererseits aber auch, daß die verdoppelten Chromosomenpaare im Laufe der Evolution verschieden stark strukturell verändert wurden. Ob sich darin aber ein unterschiedliches phylogenetisches Alter der duplizierten Chromosomen ausdrückt und die gegenwärtigen Chromosomenzahlen der Genome durch eine sukzessive Verdoppelung einzelner Chromosomen zustande kamen, wird kaum zu beweisen sein. Hypothetisch bleibt auch die zweite Möglichkeit, daß sich das „Urgenom" insgesamt verdoppelte und hernach einzelne Chromosomen wieder verlorengingen. Im letzteren Falle hätten sich die übrigen Chromosomen, die doppelt erhalten blieben, während der Evolution gegenüber formändernden Vorgängen als unterschiedlich anfällig erwiesen. Daß bei *B. campestris* ein Chromosomentyp dreimal vorkommt und hier beide Nukleolenchromosomen morphologisch sehr ähnlich sind, entspricht mehr der ersten Hypothese. Andererseits läßt sich für die zweite anführen, daß die beiden SAT-Chromosomen von *B. nigra* zu verschiedenen Strukturtypen gehören. Man müßte hier also wohl annehmen, daß dieses Genom alloploiden Ursprungs ist.

Ebenso spekulativ wären alle Bemühungen, die Ursachen der Strukturänderungen aufzuzählen, die im Laufe der Evolution dieser *Brassica*-Genome zu der heutigen unterschiedlichen Gestalt der einzelnen duplizierten Chromosomen oder gar der ganzen Genome führten. Es sei deshalb nur darauf hingewiesen, daß 1. spontane und ungerichtete Chromosomenaberrationen als evolutionistisch wirksame Faktoren aus zahlreichen Versuchen bekannt sind, während 2. GOTTSCHALK (1954b) in vergleichenden morphologischen Untersuchungen an den Pachytänchromosomen einer großen Anzahl von Arten der Familie *Solanaceae* einige langsam und gerichtet ablaufende Mechanismen aufzeigen konnte, die offensichtlich die Menge und Verteilung von Heterochromatin und Euchromatin in den Chromosomen verändern.

Summary

1. Pachytene chromosome structure of the 3 *Brassica* species *B. campestris* (genome a; $n = 10$), *B. oleracea* (genome c; $n = 9$), and *B. nigra* (genome b; $n = 8$) is described. In each of these species the same 6 chromosome types can be recognized by such structural characteristics as total length, symmetry of arms, and especially shape of the heterochromatic centromere region.

2. *B. campestris* is doubly tetrasomic for 2 chromosomes of different type and hexasomic for another chromosome type. *B. oleracea* is a triple tetrasomic, *B. nigra* a double tretasomic. The chromosome types present in increased numbers are different ones in the 3 species. The formulas for the genomes are: a = AA B C DD E FFF, c = A BB CC D EE F, and b = A B C DD E FF.

3. Secondary paring has been observed in pachytene of a triploid aac-hybrid, proving partial homology in the different genomes of some chromosomes belonging to the same structural type.

4. In each of the 3 genomes the duplicated chromosomes can be recognized by the occurrence of secondary pairing, the frequency of which depends on the degree of structural similarity. Secondary pairing is found in pachytene as well as metaphase I and II.

5. These results agree with earlier observations (see Discussion) and confirm the presence of balanced secondary polyploidy in *Brassica* derivable from a basic chromosome number $x = 6$.

Literatur

ALAM, Z.: Cytological studies of some Indian oleiferous *Cruciferae*. III. Ann. Bot. **50**, 85—102 (1936).

ALLGAYER, H.: Genetische Untersuchungen mit Gartenkohl (*Brassica oleracea*) nach Kreuzungsversuchen von RICHARD FREUDENBERG. Z. indukt. Abstamm.- u. Vererb.-Lehre **47**, 191—260 (1928).

BAJER, A.: Change of length and volume of mitotic chromosomes in living cells. Hereditas (Lund) **45**, 579—596 (1959).

CATCHESIDE, D. G.: The chromosomal relationships in the swede and turnip groups of *Brassica*. Ann. Bot. **48**, 601—633 (1934). — Secondary pairing in *Brassica oleracea*. Cytologia (Tokyo) FUJII Jub. Bd., S. 366—378 (1937).

DARLINGTON, C. D.: The behaviour of polyploids. Nature (Lond.) **119**, 390—392 (1927).

EBERLE, P.: Cytologische Untersuchungen an Gesneriaceen. I. Mitt. Die Struktur der Pachytänchromosomen, sowie eine Reihe neu bestimmter Chromosomenzahlen. Chromosoma (Berl.) **8**, 285—316 (1956). — Cytologische Untersuchungen an Gesneriaceen. II. Mitt. Die Verkürzung eu- und heterochromatischer Chromosomenabschnitte vom Pachytän bis zur Metaphase I. Ber. dtsch. bot. Ges. **70**, 323—332 (1957).

FOCKE, W. O.: Die Pflanzenmischlinge. Berlin 1881.

GOTTSCHALK, W.: Die Grundzahl der Gattung *Solanum* und einiger *Nicotiana*-Arten. Ber. dtsch. bot. Ges. **67**, 369—376 (1954a). — Die Chromosomenstruktur der Solanaceen unter Berücksichtigung phylogenetischer Fragestellungen. Chromosoma (Berl.) **6**, 539—626 (1954b). — Die Paarung homologer Bivalente und der Ablauf von Partnerwechseln in den frühen Stadien der Meiosis autopolyploider Pflanzen. Z. indukt. Abstamm.- u. Vererb.-Lehre **87**, 1–24 (1955). — Untersuchungen über die Variabilität der Struktur von Pachytänchromosomen. Ber. dtsch. bot. Ges. **71**, 381—398 (1958).

GRÖBER, K.: Multivalentbildung und „secondary association" in den Stadien der Meiosis autotetraploider Rassen der Gattung *Lycopersicon* MILL. Kulturpflanze **6**, 198—236 (1958).

HAGA, T.: Relationship of genom to secondary pairing in *Brassica*. (A preliminary note). Jap. J. Genet. **8**, 277—284 (1938a). — On genoms in the genus *Brassica*. (A collective review.) Jap. J. Genet. **14**, 74—90 (1938b)

HEILBORN, O.: The mechanism of the socalled secondary association between chromosomes. Hereditas (Lund) **22**, 167—188 (1936).

KWAN, C. C.: Inheritance of some plant characters in cabbage, *Brassica oleracea* var. *capitata*. J. Agr. Ass. China No 126—127, S. 81—127 (1934).

LAWRENCE, W. J. C.: The genetics and cytology of *Dahlia* species. J. Genet. **21**, 125—159 (1929). — The secondary association of chromosomes. Cytologia (Tokyo) **2**, 352—384 (1931).

LIMA DE FARIA, A.: Chromomere analysis of the chromosome complement of rye. Chromosoma (Berl.) **5**, 1—68 (1952).

LINNERT, G., Untersuchungen über die Cytologie polyploider Pflanzen II. Chromosoma (Berl.) **3**, 399—417 (1949). — Die Struktur der Pachytänchromosomen in Euchromatin und Heterochromatin und ihre Auswirkung auf die Chiasmabildung bei *Salvia*-Arten. Chromosoma (Berl.) **7**, 90—128 (1955).

MALINOWSKI, E.: Genetics of *Brassica*. Bibliogr. Genet. **5**, 1—26 (1929).

MIZUSHIMA, U.: Karyogenetical studies on *Brassiceae*. [Japanisch.] Tokyo: Gihodo, 1952.

MORINAGA, T.: Interspecific hybridization in *Brassica*. I. The cytology of F_1-hybrids of *B. napella* and various other species with 10 chromosomes. Cytologia (Tokyo) **1**, 16—27 (1929). — Interspecific hybridization in *Brassica*. VI. The cytology of F_1-hybrids of *B. juncea* and *B. nigra*. Cytologia (Tokyo) **6**, 62—67 (1934).

OEHLKERS, F., u. P. EBERLE: Spiralen und Chromomeren in der Meiosis von *Bellevalia romana*. Chromosoma (Berl.) **8**, 351—363 (1957).

OLSSON, G., and A. HAGBERG: Investigations on haploid rape. Hereditas (Lund) **41**, 227—237 (1955).

PEASE, M. S.: Genetic studies in *Brassica oleracea*. J. Genet. **16**, 363—385 (1926).

RAMANUJAM, S.: A haploid plant in toria (*Brassica campestris* L.). Proc. Indian Acad. Sci. **14**, 25—34 (1941).

—, and D. SRINIVASACHAR: Cytogenetic investigations in the genus *Brassica* and the artificial synthesis of *B. juncea*. Indian J. Genet. Plant Breeding **3**, 73—88 (1943).

RICHHARIA, R. H.: Cytological investigation of *Raphanus sativus*, *Brassica oleracea*, and their F_1 and F_2 hybrids. J. Genet. **34**, 19—44 (1937a). — Cytological investigation of 10-chromosome species of *Brassica* and their F_2-hybrids. J. Genet. **34**, 45—55 (1937b).

RIEGER, R.: Inhomologenpaarung und der Meioseablauf bei haploiden Formen von *Antirrhinum majus* L. Chromosoma (Berl.) **9**, 1—38 (1957).

SHIBATA, K.: Cytogenetical studies of F_1-plants of *Brassica pekinensis* (4×) × *B. napus*. Kromosomo (Tokyo) **31**, 1029—1035 (1956).

SIKKA, K.: Cytogenetics of *Brassica* hybrids and species. J. Genet. **40**, 441—509 (1940).

THOMPSON, K. F.: Production of haploid plants of marrow-stem kale. Nature (Lond.) **178**, 748 (1956).

U, N.: Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jap. J. Bot. **7**, 389—452 (1935).

YARNELL, S. H.: Cytogenetics of the vegetable crops. II. Crucifers. Bot. Rev. **22**, 81—166 (1956).

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CONFIGURATIONS RESULTING FROM ISO-CHROMATID AND ISO-SUBCHROMATID UNIONS AFTER MEIOTIC AND MITOTIC PROPHASE IRRADIATION*

By

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With 13 Figures in the Text

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Introduction

In an earlier publication (WILSON, SPARROW, and POND 1959) the authors have considered the nature of the chromosomal configurations derived from the x irradiation of various stages of meiosis in *Trillium erectum* L. The present paper is partly theoretical but is based on observations of analyzable aberrations and configurations interpreted as arising from iso-chromatid and iso-subchromatid unions in irradiated meiotic and mitotic chromosomes of *Trillium*. It seems highly probable, however, that our conclusions may be applicable in general to other organisms with well defined chromosomes.

Iso-chromatid Unions

a) General Considerations

When cells are exposed to ionizing radiation at stages of meiosis from pachytene to diplotene, or later, one of the most characteristic of the resulting configurations is an anaphase bridge and fragment similar in appearance to that found at meiosis in inversion heterozygotes. Theoretically, such configurations can arise in two ways: (1) by breakage of and transverse union between homologous chromatids and (2) breakage of and transverse (iso-chromatid) union between sister chromatids (SAX 1950, 1957; LEA 1955; MITRA 1958). In both cases, the position of the union relative to the kinetochore and chiasmata dictates not only the kind of configuration expected but also the stage of the nuclear cycle at which it will be most readily recognized. The first hypothesis appears to have been more or less generally adopted. However, it has no theoretical advantages over the second and in *Trillium* the average distance between homologous chromatids makes it comparatively unlikely that they would be broken at the same locus by a single ionization track

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produced by x rays (Lea 1955). Also, if they are broken, union is less probable because of greater distance than is union of broken sister chromatids. Therefore, for purposes of this discussion the first hypothesis will be excluded. It should be pointed out, however, that the general argument and conclusions presented here are not dependent upon which of these hypotheses is adopted.

b) Configurations Derived from Single Iso-chromatid Unions in Meiosis

The potential for the formation of diagnostic chromosome configurations from single iso-chromatid unions in a half-bivalent may be explored fully by considering the three following cases: kinetochore-union-chiasma (a or a_1); kinetochore-chiasma-union (b or b_1) and kinetochore-chiasma-chiasma-union (c or c_1) (see Fig. 1, table, lines 1—4). In the first case, the result will be a fragment and a centric loop (Fig. 7) which becomes a dicentric at second anaphase. In the second case, the result will be a bridge and fragment of the classic type at first anaphase. In the third case, if the chiasma pair is either a 2- or 4-strand double (Fig. 1, I, II), the expected configurations will be the same as for case 1 while, if the chiasma pair is a 3-strand double (Fig. 1, III), expectation will be the same as for case 2. The relative frequency of first and second anaphase bridges, therefore, is determined by the number and pattern of chiasmata between the kinetochore and points of union.

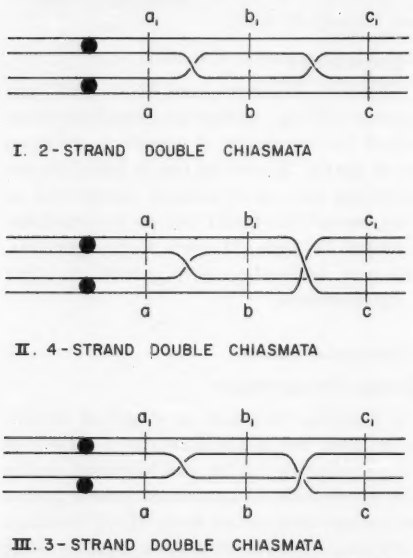

Fig. 1. Diagrams of meiotic prophase bivalents showing possible positions of iso-chromatid unions in combination with chiasmata

c) Configurations Derived from Double Iso-chromatid Unions in Meiosis

With respect to two iso-chromatid unions distributed along a half-bivalent arm, the basic rearrangement potential is provided by considering the following in various combinations: (1) unions in the same

Table. *Cytological Configurations from the Various Combinations of Chiasmata and Iso-chromatid Unions Shown in Fig. 1*

	Line No.	Bivalent Number (Fig. 1)	Positions of Iso-chromatid Unions (from Fig. 1)	Bridge		Fragment	Triplicated Segment[1]	Double Triplicated Segment[1]
				A I	A II			
Single Unions	1	I, II, III	a or a_1		+	+		
	2	I, II, III	b or b_1	+		+		
	3	I, II	c or c_1		+	+		
	4	III	c or c_1	+		+		
Double Unions	5	I, II, III	a&b, a_1&b_1, a&b_1 or a_1&b		+	+	+	
	6	I, II, III	b&c, b_1&c_1, b&c_1 or b_1&c	+		+	+	
	7	I	a&c or a_1&c_1		+	++[2]		
	8	I	a&c_1 or a_1&c		++	++		
	9	II	a&c or a_1&c_1		++	++		
	10	II	a&c_1 or a_1&c		+	++[2]		
	11	III	a&c, a_1&c_1 a&c_1 or a_1&c		+	+	+	
Triple Unions	12	I	a&b&c, a_1&b_1&c_1, a&b_1&c or a_1&b&c_1		+	+		+
	13	I	a&b&c_1, a_1&b_1&c, a&b_1&c_1 or a_1&b&c		+	+	++	
	14	II	a&b&c, a_1&b_1&c_1, a&b_1&c or a_1&b&c_1		+	+	++	
	15	II	a&b&c_1, a_1&b_1&c, a&b_1&c_1 or a_1&b&c		+	+		+
	16	III	a&b&c, a_1&b&c, a_1&b_1&c_1 or a&b_1&c		+	+	++	
	17	III	a&b_1&c_1, a_1&b&c_1, a&b&c or a_1&b_1&c		+	+		+

[1] The middle segment of the triplicated portion is in the form an inversion.
[2] These cells have one acentric fragment plus one acentric ring.

or opposite half-bivalents and (2) unions separated by no chiasmata one chiasma or two chiasmata. Only two of the several possible combinations offer anything basically different from single unions. If two unions are separated by a single chiasma and regardless of whether they are in the same or opposite half-bivalents, we expect a fragment and either a first or second anaphase bridge (depending on the position of the most proximal union relative to the kinetochore and proximal chiasma) and a chromatid with a triplicated segment, the middle portion of which is inverted (Fig. 1, table, lines 5—6, and Figs. 2 and 5a). If the unions are separated by a pair of chiasmata and no chiasmata intervene between the kinetochore and the most proximal union, we expect the following: where the chiasma pair is a 2- or 4-strand double (Fig. 1, I and II), there will be second anaphase bridges and fragments, the number and kind of which depend upon whether the two unions are in the same or opposite half-bivalents (table, lines 7—10); where the

chiasma pair is a 3-strand double (Fig. 1, III), we obtain a second anaphase bridge (or a first anaphase chromatid loop) and fragment as well as a chromatid with a triplicated segment (table, line 11, see also

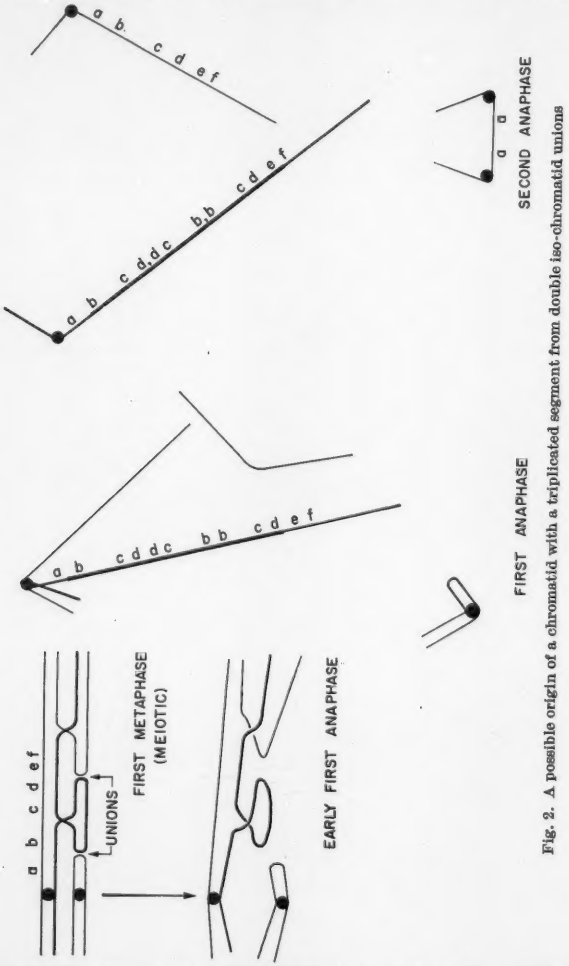
a b c d e f
a b c d d c b b c d e f
a b c d d c b b c d e f
a a
SECOND ANAPHASE
FIRST ANAPHASE
a b c d e f
UNIONS
FIRST METAPHASE (MEIOTIC)
EARLY FIRST ANAPHASE

Fig. 2. A possible origin of a chromatid with a triplicated segment from double iso-chromatid unions

Figs. 6—8). By the microspore metaphase the latter chromatid (with triplication) would be expected to occur as an unusually long monocentric chromosome. These have been seen frequently, especially in tetraploid microspores (Figs. 10—13).

d) Configurations Expected from Triple Iso-chromatid Unions in Meiosis

Although triple unions (table, lines 12—18) combined with chiasmata offer nothing fundamentally different from the cases already discussed,

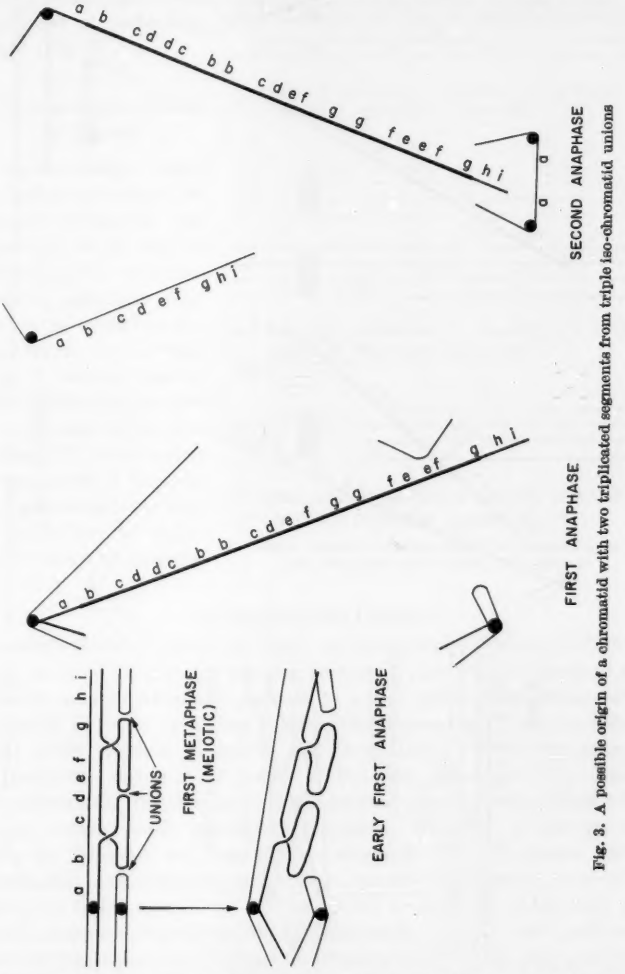
a b c d d c b b c d e f g g f e e f g h i
a a
SECOND ANAPHASE
a b c d e f g h i
a b c d d c b b c d e f g g f e e f g h i
FIRST ANAPHASE
a b c d e f g h i
UNIONS
FIRST METAPHASE (MEIOTIC)
EARLY FIRST ANAPHASE

Fig. 3. A possible origin of a chromatid with two triplicated segments from triple iso-chromatid unions

an example will serve to show how multiple replication might occur. If we consider the case of three unions with each pair separated by a chiasma, we find that we can derive, in addition to the usual bridges

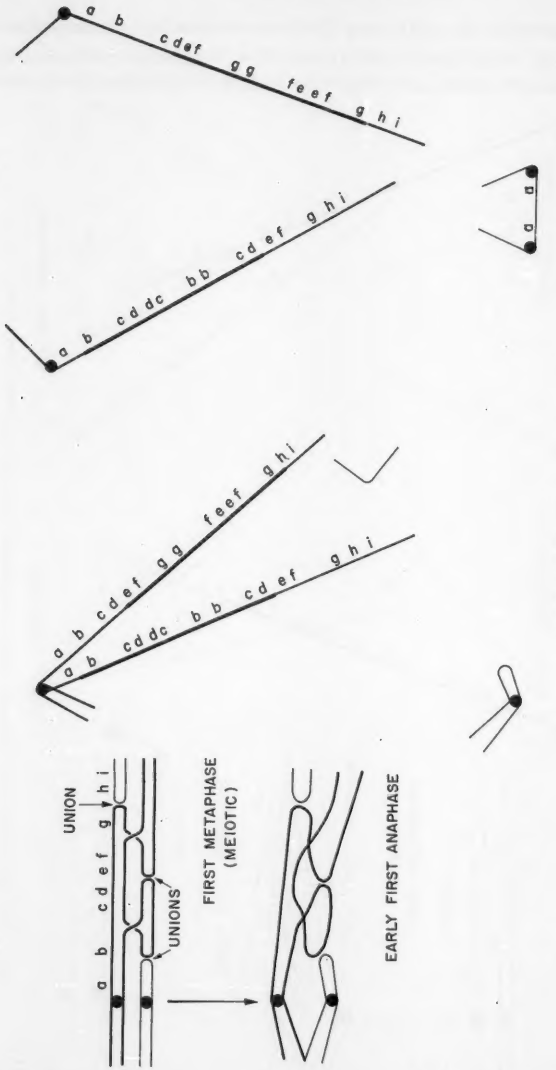

Fig. 4. A possible origin of two chromatids each with a triplicated segment from triple iso-chromatid unions

and fragments, either two chromatids with triplicated segments (table, lines 13, 14, 16) or one with two such segments (table, lines 12, 15, 18) depending on the distribution of breaks in the two half-bivalents and

on the types of chiasma pairs. If the normal order were *abcdefghi*, then with breaks between *a* and *b* and *d* and *e* the former case would produce the two chromatids *abcddcbbcdefghi* and *abcdefggfeefghi* (Fig. 4) and the latter would give one chromatid *abcddcbbcdefgg feefghi* (Fig. 3).

e) Iso-chromatid Unions in Mitosis

Iso-chromatid unions in prophase of mitosis also produce dicentrics and fragments which will be revealed at the anaphase following induction. Multiple unions within an arm may result in acentric rings of various sizes as well as dicentrics but there is no potential for the production of monocentric chromatids with triplicated segments as there is in the pachytene to diplotene bivalent of meiosis.

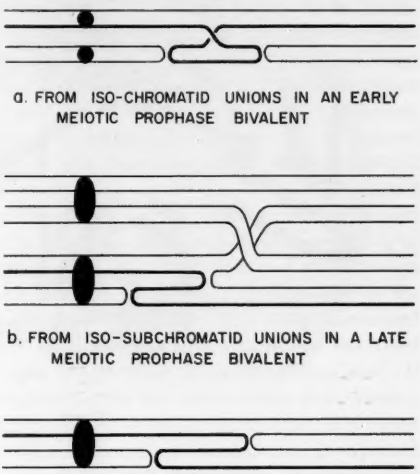
a. FROM ISO-CHROMATID UNIONS IN AN EARLY MEIOTIC PROPHASE BIVALENT
b. FROM ISO-SUBCHROMATID UNIONS IN A LATE MEIOTIC PROPHASE BIVALENT
c. FROM ISO-SUBCHROMATID UNIONS IN A LATE MITOTIC PROPHASE CHROMOSOME

Fig. 5. Various possible origins of chromatids or subchromatids with triplicated segments

Iso-subchromatid Unions

a) Configurations Derived from Single Iso-subchromatid Unions in Meiosis

It has now been quite well demonstrated that when a bivalent at diplotene to late diakinesis is exposed to x rays, pairs of half-chromatids may break followed by unions between the broken ends (Crouse 1954; Mitra 1958; Wilson, Sparrow, and Pond 1959). Where the unions are transverse and between halves of the same chromatid (i.e., sister half-chromatids), the diagnostic configuration is a dicentric which will not be revealed until microspore anaphase. However, if the halves belong to different but associated chromatids (i.e., non-sister half-chromatids), then either transverse or reciprocal interchange unions will result in a 2-side-arm bridge which will be revealed at either first or second anaphase depending on the chiasmata number and pattern. Since the consequences of single iso-subchromatid unions per bivalent have been explored in a previous paper (Wilson, Sparrow, and Pond 1959), we will consider below only some of the more important consequences of multiple unions.

Fig. 6

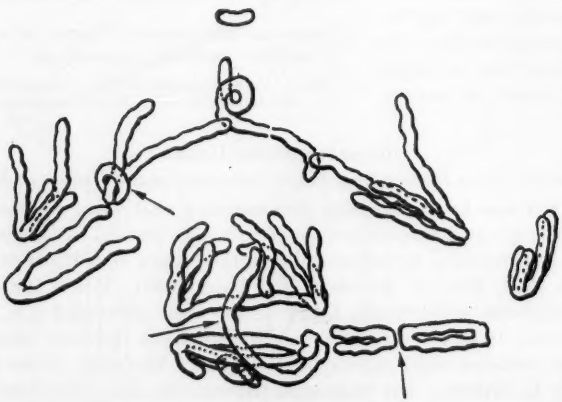

Fig. 7

Fig. 6 and 7. Fig. 6. First meiotic anaphase in *Trillium erectum* showing various aberrations induced by 50 r of x rays at early pachytene. × 1700 (see also Fig. 7). **Fig. 7.** Drawing of cell illustrated in Fig. 6. Indications of iso-chromatid breakage and union can be seen (short arrows). The rings would be expected to form dicentrics at second anaphase. The possibility of a triplicated segment is suggested by the over-long chromatid arm at bottom center (long arrow)

b) Configurations Derived from Double Iso-subchromatid Unions in Meiosis

When the half-bivalent is in the 4-strand condition and chromatids are closely associated as appears to be the case from diplotene to late diakinesis, double iso-subchromatid unions may be of three kinds: (1) both unions between sister half-chromatids, (2) both between non-sister half-chromatids or (3) one of each. The consequences of these unions could be explored in the same manner as for the consequences of meiotic iso-chromatid unions described above (table, Fig. 1); however, briefly they can be summarized as follows: in the first case, we expect in addition to fragments, rings and dicentrics of various sizes which will be revealed at microspore division; in the second case, we expect meiotic 2-side-arm bridges with various complications depending in part on chiasmata distribution. Certain cases here (pseudo side-arm bridges) can produce monocentric triplicated half-chromatids. If these escape rupture at meiotic anaphase, they could produce chromatids at the microspore division with triplicated segments. The other chromatid normally would be expected to be of standard length. In the third case, expectation depends upon which kind of union is proximal. If the proximal union is between non-sister half-chromatids, some version of the 2-side-arm bridge is expected, but if the proximal union is between sister half-chromatids some type of branched chromosome is expected (Fig. 5b). In either case, one of the half-chromatids should have a triplicated segment similar to that described above. In the former case, however, such a half-chromatid normally would be disrupted by breakage of the bridge as stated above for case two. In the latter case, it can reasonably be expected to be carried to the microspore division where its presence should be indicated by a prophase or metaphase chromosome with chromatids of different lengths. Such a configuration would not necessarily be completely diagnostic since it conceivably could be produced in other ways; e.g., by a half-chromatid deletion or duplication.

c) Configurations Derived from Iso-subchromatid Unions in Mitosis

What has been said for iso-subchromatid unions in diplotene to diakinesis of meiosis applies equally well to stages of mitosis where the chromosomes are effectively four-partite. At the stage or stages in which it is possible to produce 2-side-arm bridges (and this seems to be rather late in prophase), it should also be possible with two transverse unions to produce a half-chromatid with a triplicated segment which would be revealed as a branched chromosome at anaphase and an over-long chromatid at the X_2 prophase or metaphase. If it survives to X_3 it should appear at metaphase as a longer than average monocentric chromosome (Fig. 5c).

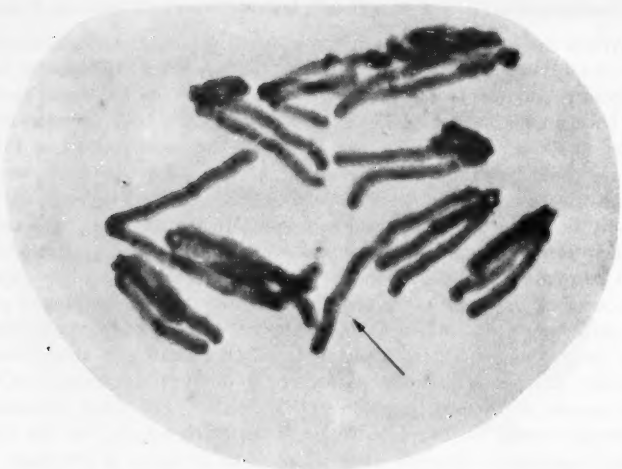

Fig. 8. First meiotic anaphase in *Trillium erectum* after 50 r of x rays at early pachytene. The chromatid with the very long arm (arrow) may have resulted from double iso-chromatid breakage and union within a bivalent. Such a chromatid would be expected to appear as a very long monocentric chromosome at microspore metaphase. × 1700

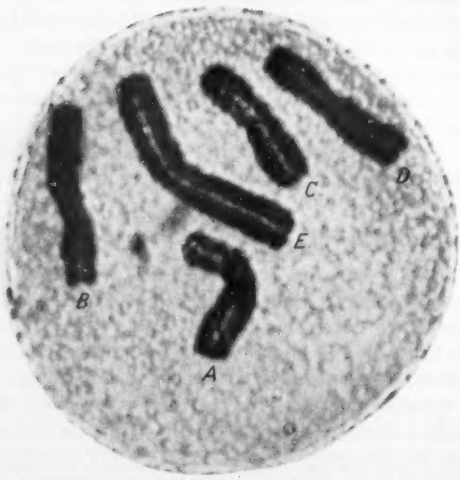

Fig. 9. A normal haploid microspore metaphase in *Trillium erectum*. The haploid number of five chromosomes can be seen and each chromosome can be distinguished readily. ×1700

More than two iso-subchromatid unions per arm in either meiosis or mitosis are not expected to produce configurations of types not implied in the cases already discussed so will not be further considered here except to point out that consideration of multiple unions of various kinds can lead to a considerable array of complex fragments including rings of various types and probably also knots.

Discussion

It now seems clear that both iso-chromatid and iso-subchromatid unions of broken ends occur after irradiation of suitable stages of meiosis or mitosis. Certain of these unions and combinations are of considerable cytological interest and may also have important genetic consequences. When the bivalent at pachytene to diakinesis is exposed to adequate amounts of ionizing radiation, the expected diagnostic configurations are a bridge and fragment or a 2-side-arm bridge, depending upon the number of independent strands per bivalent. Long triplicated chromatids may also result. The stage at which the bridges are expected to be revealed depends on the chiasmata number and on their pattern. Where such configurations are used as an index of radiosensitivity of different stages this factor should be taken into account. This and related matters will be discussed in a later paper.

From the genetic point of view, the most important implication of the types of unions discussed is the possibility of obtaining monocentric chromatids (and eventually chromosomes) with triplicated segments and without deficiencies. Such chromosomes in themselves would represent new genetic arrangements of considerable interest. Also, subsequent modification by suitable translocation or, possibly, unequal or abnormal crossing over with normal homologues could lead to a whole gamut of rearrangements and thus such chromosomes, if viable, should have, in theory at least, considerable evolutionary potentiality similar to that attributed to duplications (see SWANSON 1957, p. 480—485). They might also be of value in practical plant breeding in certain cases (see SPARROW 1956).

A major question relating to the likelihood of the above proposed rearrangements seems to be dependent upon the production of a sufficient number of breaks and unions of the type required. There seems little doubt of this in our material, since the fragment yield from a dose of 100 r averages about 28 per cell, which, based on chromosome length, corresponds to about 4 for each arm of the E and the long arm of the A. If we assume that there were no open ends and that reciprocal interchanges and restitution injuries were random possibilities, the 4 fragments per arm would represent at least 12 original breaks since only the iso-union, which should represent $^1/_3$ of the possibilities, would be detectable.

Fig. 10

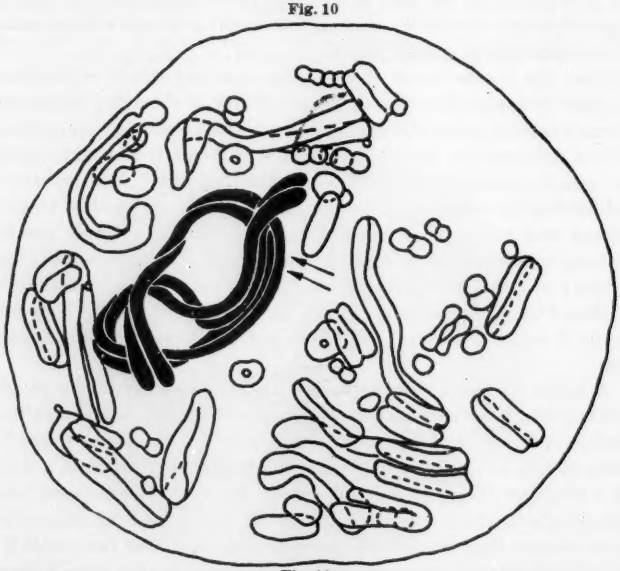

Fig. 11

Fig. 10 and 11. Fig. 10. Tetraploid microspore metaphase with a high degree of fragmentation resulting from irradiation with 100 r of x rays at first meiotic metaphase. ×1700 (see also Fig. 11). Fig. 11. Drawing of tetraploid metaphase illustrated in Fig. 10. The chromosome drawn in black appears to be monocentric and is about 4× the length of the longest chromosome in a normal complement as shown in Fig. 9. It could include a triplication (see text). (The two arrows indicate the approximate position of two small fragments located under the long chromosome. They were omitted for reasons of clarity)

The probability that any given chiasma would separate at least one pair of iso-chromatid unions is obviously fairly high. If we take as a model a bivalent arm having a chiasma approximately centrally placed and an average of 6 breaks along each half-bivalent, the probability that two iso-chromatid unions will be separated by a chiasma and therefore give rise to a triplication is approximately 1 in 5. Since a *Trillium* cell can be considered to contain 8 such chromosome arms, the probability of getting at least one triplication would amount almost to a certainty with the level of breakage postulated above.

Although undoubtedly tedious in execution, a number of genetic tests for the presence of a chromosome with a triplicated segment of the type discussed could almost certainly be devised. Any such test would have to be carried out with a suitable selection of genetic markers on material irradiated at a suitable stage and exposed to a dose which would be high enough to produce the minimum number of breaks required but not so great as to result in an unduly high degree of fragmentation. In general, meiosis from pachytene to diakinesis would seem to be the most suitable time for treatment not only because a large number of cells would be in a suitable stage simultaneously but also because the successive chromosome geometries are favorable for the derivation of chromosomes with triplicated segments. Cytological evidence favoring the hypothesis has already been obtained from our experiments on anthers of *Trillium erectum* irradiated over the pachytene to diakinesis range. Tissue undergoing mitosis would be suitable only where there was considerable synchrony as otherwise the yield might be too low to be practical. The first microspore division of some plants might offer an appropriate target.

The irradiation of meiotic cells before they reach first anaphase with x-ray doses in the 100—200 r range has produced a rather large number of tetraploid microspores through failure of both first and second anaphase spindles (SPARROW 1944; SPARROW and SPARROW, in press). At first microspore division, such cells characteristically show large numbers of rather small fragments, both rod and ring, including occasional knots, plus one or more very long chromosomes which may be either centric or acentric (Figs. 10—13). The diagrams by KURABAYASHI (1958) of first meiotic metaphase configurations in *Trillium kamtschaticum* following irradiation suggest that very long chromosomes could be produced at microspore metaphase in this species of *Trillium* also. Extraordinarily long microspore metaphase chromosomes also have been observed by HAQUE (1953) in *Tradescantia* following meiotic prophase irradiation but did not receive special comment. Such long chromosomes may include an unknown percentage with triplicated segments as expected on the hypothesis proposed in this paper. (Short triplications

Fig. 12. Tetraploid microspore metaphase showing many fragments resulting from irradiation with 100 r of x rays at first meiotic metaphase. ×1700. The long chromosome across the middle of the cell is about 3.5 × as long as the longest chromosome of a normal complement and could include a triplication (see text)

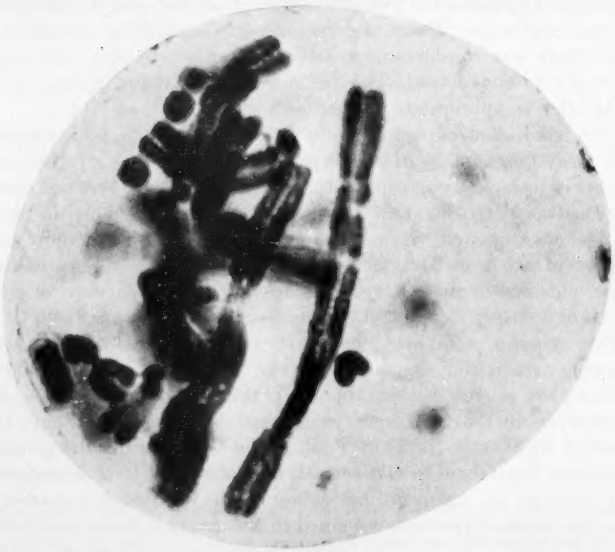

Fig. 13. Tetraploid microspore metaphase resulting from irradiation with 100 r of x rays at first meiotic metaphase. ×1700. The long chromosome at the right side of the group is about 2 × the length of the longest chromosome of a normal complement

would not be detectable cytologically in such material but might be genetically at least as important as the longer form.)

If we are corrent in our assumptions, then the chromosomes in the bivalent condition at meiosis and in the 4-strand stage at mitosis represent a potential for genetic change and structural modification not present at any other time (Fig. 5). The possibilities presented above seem to us to be sufficiently significant to warrant further investigation.

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Summary

By making use of the chromosomes of *Trillium erectum* as a model, potential and actual configurations arising from presumed iso-chromatid and iso-subchromatid unions after irradiation of meiotic or mitotic prophase have been studied and analyzed. Diagrams and photographs of various recognizable types of chromatid or subchromatid rearrangements are presented. A minimum of two iso-chromatid unions within an arm of a single chromosome in meiotic prophase, if separated by a single chiasma, can give rise to a monocentric chromosome with a triplicated segment, the middle portion of which is an inversion. A minimum of two iso-subchromatid breaks within an arm at either meiotic or mitotic prophase also can result in the production of a monocentric chromosome containing a triplicated segment. The stage of appearance of dicentrics or bridges arising from chromatid or subchromatid unions in meiotic prophase is influenced by chiasma number and pattern and by the number of strands per chromosome or chromatid. Some of the rearrangements described may have genetic and evolutionary implication of considerable potential importance which has not been recognized previously.

Literature cited

Crouse, H. V.: X-ray breakage of lily chromosomes at first meiotic metaphase. Science **119**, 485—487 (1954).

Haque, A.: The irradiation of meiosis in *Tradescantia*. Heredity **6** (Suppl.), 57—75 (1953).

Kurabayashi, M.: Configurations taken by x-ray induced heteromorphic chromosome pairs at M_1 in the PMCs of *Trillium kamtschaticum* Pall. Cytologia (Tokyo) **23**, 317—333 (1958).

Lea, D. E.: Actions of radiations on living cells. London: Cambridge University Press 1955. xiv + 416 pp.

Mitra, S.: Effects of x rays on chromosomes of *Lilium longiflorum* during meiosis. Genetics **43**, 771—789 (1958).

Sax, K.: The effect of x rays on chromosome structure. J. cell. comp. Physiol. **35** (Suppl. 1), 71—81 (1950). — The effect of ionizing radiation on chromosomes. Quart. Rev. Biol. **32**, 15—26 (1957).

Sparrow, A. H.: X-ray sensitivity changes in meiotic chromosomes and the nucleic acid cycle. Proc. nat. Acad. Sci. (Wash.) **30**, 147—155 (1944). — Cytological changes induced by ionizing radiations and their possible relation to the production of useful mutations in plants. In: Work Conference on Radiation Induced Mutations, Brookhaven National Laboratory, Upton, N.Y. 1956, pp. 76—113.

—, and R. C. Sparrow: The relationship between induced polyploidy and x-ray dosage in *Trillium erectum* L. (in press).

Swanson, C. P.: Cytology and cytogenetics. Englewood Cliffs, N. J.: Prentice-Hall 1957. x+ 596 pp.

Wilson, G. B., A. H. Sparrow and V. Pond: Sub-chromatid rearrangements in *Trillium erectum* L. I. Origin and nature of configurations induced by ionizing radiation. Amer. J. Bot. **46**, 309—316 (1959).

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THE STRUCTURE OF BIVALENTS IN THE HOMOPTERA AUCHENORRHYNCHA

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With 25 Figures in the Text

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Introduction

The Hemipterous insects, with their comparatively large chromosomes, have been used for studies of cytogenetic problems since the initiation of this branch of science. However, although useful for many purposes, the Hemipterans are generally ill-suited for work on two critical phases of meiosis, chiasma formation and the preceding pairing. This is because at zygotene there is a tendency for "synizesis", and because a prolonged diffuse stage often intervenes and interferes with observations at pachytene and diplotene.

As regards somatic divisions, the endomitotic tendencies shown by many tissues of Hemipterans have been exploited by a number of students, notably GEITLER (1953). Since the Hemipterans seem to lack any tendency towards somatic pairing, the analysis of polyploid nuclei usually does not give detailed information on chromosome structure.

The Heteropterans have played a key role in the development of the concept of differential polyteny in relation to evolution within families by the SCHRADER'S and others (SCHRADER and HUGHES-SCHRADER 1956). Similar work has been done on sternorrhynchous Homopterans (HUGHES-SCHRADER 1957). The present paper, with its emphasis on the bivalent structure in the auchenorrhynchous group, is also an attempt to extend polyteny (DNA-) studies to cover this section of the Homoptera. For this purpose, a leafhopper species with chromosomes of a size typical of the family *Cicadellidae, (Graphocephala coccinea)*, is compared with another member of the family, a primitive species with unusually large chromosomes *(Xerophloea viridis)*.

I wish to express my gratitude to Professors M. DEMEREC and A. W. POLLISTER, of Cold Spring Harbor and New York, respectively, in whose laboratories the present work was done. At Cold Spring Harbor I worked in the section headed

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by Dr. B. P. KAUFMANN and, during his vacation, by Dr. HELEN GAY, both of whom I wish to thank for their helpfulness and technical advice. I also stand in debt to Dr. BETTY C. MOORE, who taught me the cytophotometric technique. Dr. J. C. PALLISTER, at the American Museum of Natural History, was very helpful in providing me with the equipment necessary for determining *X. viridis*, for which I express my thanks.

I wish to thank Professor F. SCHRADER for some very helpful suggestions. I am grateful to Mrs. JEAN MARGARET PERTTUNEN, B. Sc., who has checked the English of my manuscript.

Material and methods

The material of the present study was collected partly in Finland, at the Tvärminne Zoological Station, partly in the United States, at Cold Spring Harbor. Observations were made on the following four species: *Ulopa reticulata* L. and *Eupelix cuspidata* F. from Tvärminne, and *Xerophloea viridis* FABR. and *Graphocephala coccinea* FORST. from Cold Spring Harbor. All are members of the large family *Cicadellidae*, the respective subfamilies being: *Ulopinae*, *Eupelicinae*, *Ledrinae* and *Cicadellinae*. The identification of *X. viridis* was based on the observation that the individuals studied matched specimens of this species at the American Museum (rather than those of *X. major*), while the genitalia of the individuals corresponded with the resp. figures by BEIRNE (1956). According to OMAN (1949), the genus *Xerophloea* may be in need of revision.

Sections were prepared of all the species, but the analysis of chromosome structure rests mainly upon Benda-Feulgen, aceto-alcohol-Feulgen, and aceto-orcein squashes.

Testes from three *G. coccinea* males and one *X. viridis* male were prepared for the cytophotometric part of this work. The material was fixed in Carnoy (absolute alcohol-chloroform-acetic acid; 6:3:1) and subsequently treated according to the procedure of LEUCHTENBERGER (1958). The *Xerophloea* sections and sections from one *Graphocephala* individual were mounted together on the same slide. This was done to ensure identical treatment of the two species, and for the same reason sectioning was done at the same thickness (10 μ). However, as in both species the nuclei are packed rather tightly at meiotic prophase, only very few *G. coccinea* late pachytene nuclei were not overlapped by other nuclei (nuclear diameter at this stage about 7.5 μ), while scarcely sufficient *X. viridis* nuclei at the same stage were uncut (nuclear diameter about 11.5 μ). The testes of the other two *Graphocephala* males were cut at 8 μ, and sections from different males mounted on separate slides. The attempt to use squashed *Xerophloea* material for cytophotometry was abandoned because the great variations in photometric area — nuclei at the same stage flattened to very different degrees —, introduced a wide margin of error. The DNA values obtained with squashed nuclei were generally distinctly higher than those recorded with sectioned material.

The two-wavelength method was used throughout, with λ 2 at 555 mμ and λ 1 at 489 mμ *(Graphocephala)* or 487 mμ *(Xerophloea)*. The extinction curves of both species run very steeply in the halfway (λ 1) region, especially the curve for *Xerophloea*. The analysis is thus very sensitive to choice of wavelengths. Several extinction curves were run for *X. viridis* pachytene nuclei, but considerable deviations from $E\lambda 2 = 2E\lambda 1$ still produced exceptional values. Therefore, after λ 2 had been fixed, and λ 1 approximately determined, experiments to get λ 1 exactly were run and data with $E\lambda 2 > 2E\lambda 1$ were provided with a — sign, those with $E\lambda 2 < 2E\lambda 1$ with a + sign. Subsequently, λ 1 was moved until a series of 10 measurements gave an equal or nearly equal number of — and + signs. It is likely that if the proper

wavelengths are thus "statistically" determined, the two-wavelength method yields reliable means despite the very steeply rising extinction curves (high extinctions), even when the spread of the DNA values is considerable. (The present cytophotometric problem being very simple, the two-wavelength method was used in the original form, although the curves for *Graphocephala* and *Xerophloea* were of slightly different shapes, and some of the extinctions for *Xerophloea* somewhat exceeded 0.6:)

Extinction curves were also run for the controls (unhydrolyzed, Feulgen-stained slides). The peak extinction in the controls was about 3% of the peak extinction in hydrolyzed (optimum at 8 minutes) sections. Thus, the Feulgen reaction was not quantitative in the sense that only HCl-hydrolyzed DNA is expected to take up the stain. It may be that some cold hydrolysis occurs in the acid Carnoy fixative. The slight flaw in the quantitativeness of the staining procedure does not affect the relative and comparative data of the present investigation.

The particular apparatus used in the present work was the same setup which has been employed by the SCHRADERS and other students of the *Hemiptera* (for description of apparatus, see ANSLEY 1954).

Observations

1. Cytological observations

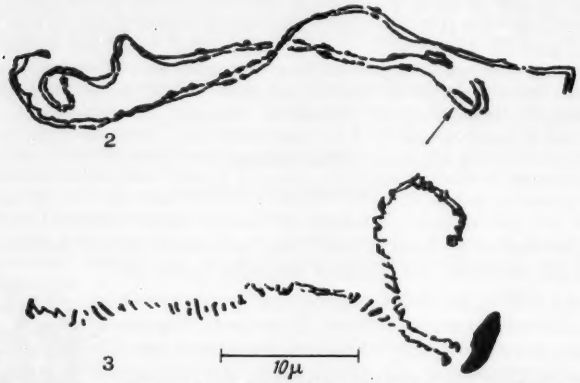
As the chromosome cytology of *Graphocephala coccinea* has been described earlier by BORING (1907), and an account of the chromosomes of *Ulopa reticulata* and *Eupelix cuspidata* given by HALKKA (1959), the following report will concentrate upon *Xerophloea viridis*. Since *Ulopa* and *Eupelix* are genera rather closely related to *Xerophloea*, comments on differences and similarities in the cytology of the three species are included. This implies somewhat more than a recapitulation of the data published earlier, as slides containing *Ulopa* and *Eupelix* material were brought over from Finland, to make possible direct comparison under the same microscope.

Fig. 1. *Xerophloea viridis*. Spermatogonial metaphase with 20 chromosomes. The largest one is the X, while the Y is one of the three other large chromosomes

Although the emphasis of the present work lies heavily on germ line cells, a few words about somatic tissues seem appropriate. In the Malpighian tubules of *X. viridis*, the nuclei attain a very large size, the largest measuring about 90 μ in diameter. The degree of ploidy is evidently high, tentative comparisons of volumes of pachytene and Malpighian tubule nuclei suggesting 64-ploidy at least. There seems to be no tendency towards somatic pairing.

In the oogonial and spermatogonial prophases, the chromosomes of *X. viridis* display a variety of spiralization patterns in the different cell generations. The chromosomes are fairly large but neither at prophase, nor at metaphase are there any indications of the presence of a localized centromere. At metaphase, two of the ten chromosome pairs are distinctly larger than the others (Fig. 1). The largest chromosome is probably the X, while the Y is about equal in size to the large autosomes.

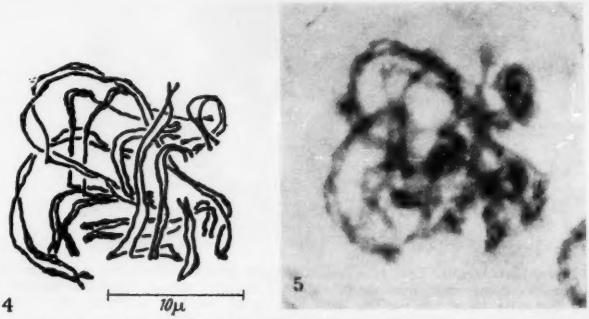
At the earliest stages of the meiotic prophase the tangled mass of chromosomes, already polarized, shows no sex chromosome heteropycnosis. This conforms with experiences of other leafhoppers with the XY type of sex determination, most of which are without heteropycnosis at leptotene. At zygotene, unpaired and paired sections are seen in the forming bivalents, but the details of pairing remain veiled in the maze of the dense early bouquet. The high frequency of free ends suggests, however, that pairing is seldom initiated at all simultaneously at both ends.



Figs. 2 and 3. Pachytene bivalents in *Xerophloea viridis* (Fig. 2) and *Ulopa reticulata* (Fig. 3). In the *Xerophloea* pachytene, the XY-pair and the large autosome pair is drawn. The formation indicated by an arrow is possibly the formerly univalent X chromosome which has retained some heteropycnotic tendency after having been attached to an autosome. In the *Ulopa* pachytene, the heteropycnotic, univalent X chromosome and two long autosome bivalents (bouquet loops) are shown. Some sections of the autosomes appear banded, some other sections displaying a continuous spiral

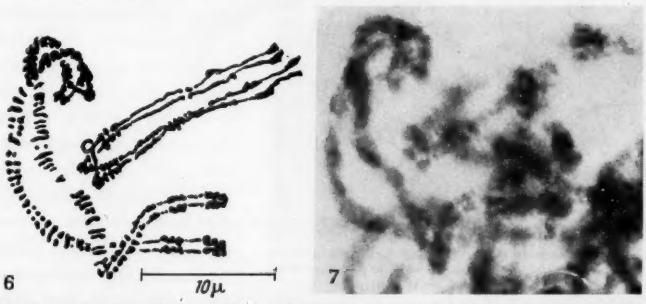
Beginning with pachytene, all the details of bivalent behavior can be observed with considerable ease in *Xerophloea*, as well as in *Ulopa* and *Eupelix*. The pachytene bouquets show beautiful, wide loops, which often burst open on being squashed and expose the structure of the bivalents for close observation. At this stage the longest bivalent of *Ulopa* is about 35 μ long, that of *Eupelix* about 50 μ, and that of *Xerophloea* about 50—60 μ long. In *Xerophloea* (and *Eupelix*) the chromosomes display typical knotlike chromomeres at this stage (Fig. 2). In *Ulopa* the chromosomes, although not much shorter than in the other two species, show a different mode of spiralization (Fig. 3). The chromosomes appear banded, the number of bands in the largest loop protruding from the bouquet varying between 30—50. As yet no signs of chiasma formation are to be seen in these loops.

In *Eupelix* and *Xerophloea*, at the onset of diplotene, the loops show four very thin strings with not much chromomere structure in them. The abolition of much of the structural detail seen in the chromosomes in the preceding stages may be regarded as the only rudiment



Figs. 4 and 5. *Xerophloea viridis*. A drawing and a photograph of the same diplotene bouquet with chiasmata. Three chiasmata are seen in the lower left corner of the nucleus

of the diffuse stage in these insects. The absence of any real diffuse stage makes the observation and photography of chiasmata possible. Chiasmata have been shown to occur in *Eupelix* bouquets (HALKKA 1959), and they are evidently present at this stage in *Xerophloea*, too



Figs. 6 and 7. *Xerophloea viridis*. A drawing and a photograph of the same nucleus, showing the chiasmata at the "banded" stage of diplotene. There is one almost terminal chiasma in both of the bivalents shown

(Figs. 4, 5). The interbivalent associations, typical of leafhopper diplotene and early diakinesis, prevent proper dispersal of the bivalents in squashing (no attempt was made to facilitate this by the use of chemicals). Therefore, as most of the chiasmata are seen near the ends, the very maintainers of interbivalent associations, generally not more

than two indisputable chiasmata per nucleus can be found. Probably the process of terminalization has already started at the stage shown in Figs. 4 and 5, i.e. some terminalization may occur before the chiasmata have become symmetrical. The above figures show convincingly that

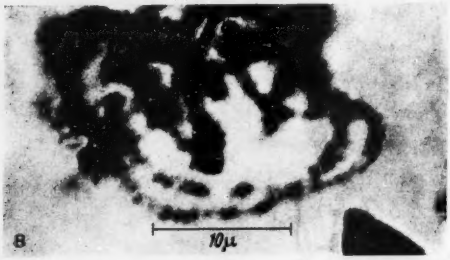


Fig. 8. *Xerophloea viridis.* Part of a large bivalent with similar banding pattern in both of the chromosomes. Sufficient stretching has exposed almost bandless regions about 2 μ long (actually these are regions with very weak bands). At right, the component chromatids are seen in the corresponding sections of the two chromosomes

the repulsion forces between the bivalent halves first become active at the bouquet stage.

In *Xerophloea* the banded stage of spiralization, attained in *Ulopa* as early as late pachytene, only commences after the disintegration of the bouquet (Figs. 6—9). As the two chromosomes of the bivalent are not uniformly stretched in the squashes, the banding does not appear exactly similar over distances greater than a few micra. Sometimes, although only over quite short distances, the four component chromatids are all seen pressed together. At these points the bands are continuous, the bands of the four chromatids placed end-to-end resulting in a very broad band not far short of the salivary chromosome structures of many Dipterans.

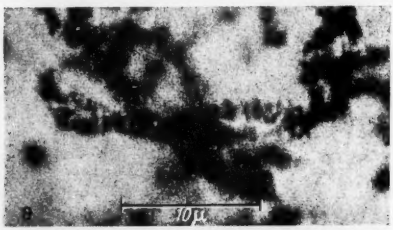
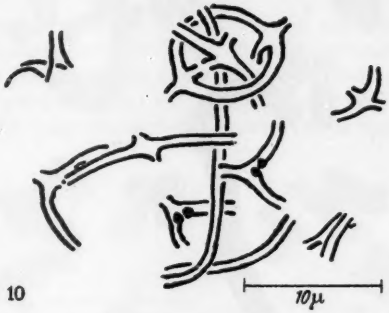


Fig. 9. *Xerophloea viridis.* A bivalent with particularly broad bands in both chromosomes. The chromosomes are loosely coiled relationally

In *Xerophloea*, the number of bands in the two biggest bivalents, the XY pair and the largest autosome pair (characterized by the frequent presence of two chiasmata) is approximately 50. In the six medium-sized chromosomes the number is around 30, and in the two smallest bivalents about 20 or less. This results in about 300 bands in the whole

set as a very rough estimate. This is of a lower order of magnitude than the number of bands in the polytene chromosomes from *Drosophila* third-stage larvae, but the degree of spiralization, not to mention the degree of polyteny, is also different in the leafhopper. Every *Xerophloea*

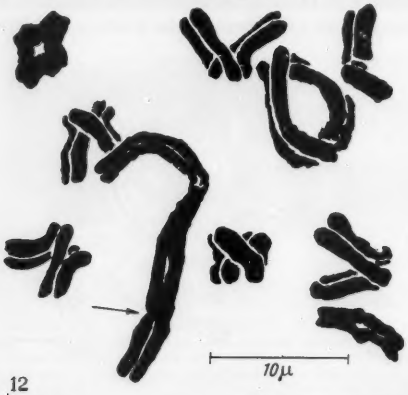


Figs. 10 and 11. *Xerophloea viridis*. A drawing and a photograph of the same diakinese nucleus. The long X-chromosome extends all the way down through the middle of the field, the Y being attached to its lower end. The large autosome bivalent has two symmetrical but unterminalized chiasmata and encircles the upper end of the X-chromosome. Inside the ring lies also one of the middle-sized bivalents

chromosome band is perhaps composed of a number of "potential band" subunits. In the three types of *Xerophloea* squashes, clear bands are seen only in aceto-orcein material.

At the next stage of spiralization, the *Xerophloea* chromosomes show a soft, "hairy" outline. This stage of spiralization is reached in late pachytene in *Ulopa*, but at about the same stage of diplotene in *Eupelix*. At diakinesis, the appearance of the bivalents in aceto-orcein squashes suggests the presence of a major-coil type spiral structure (Fig. 13).

In *Eupelix* the chiasmata terminalize a little earlier than in *Xerophloea*. The frequency of chiasmata is about the same in the two species,



Figs. 12 and 13. *Xerophloea viridis*. A drawing and a photograph of the same diakinese nucleus. Most of the chiasmata have not yet terminalized, and many have not even become symmetrical. The invagination in the supposed X-chromosome, indicated by an arrow, probably marks the point where an X-element has become attached to what was an autosome. Due to movements of chromosomes in the non-permanent preparation, there are differences between the drawing and the photograph in this as well as some other pairs of figures

i.e., one chiasma per bivalent except in one, two or at most three bivalents (Figs. 10—13). In *Eupelix*, attraction between the non-chiasmate ends makes rings even of bivalents which, according to true

definition, are rods. This tendency is almost non-existent in *Xerophloea*, but in this species interbivalent associations are seen at diakinesis. The low frequency of chiasmata fits in with what could be observed of pairing at zygotene, there being probably only a single pairing block in most of the bivalents.

The chromosome complements of *Eupelix* (8 autosome pairs and a univalent X chromosome) and *Ulopa* (9 autosome pairs and the X univalent) have been described earlier (HALKKA 1959). *Graphocephala* has 11 pairs of autosomes and an X. *Xerophloea* has 9 pairs of autosomes plus an XY pair. The sex chromosome pair is apparently a neo-

Figs. 14 and 15. *Xerophloea viridis*. First maturation division metaphase plates, with the X and the Y completely separated (Fig. 14) and in an abnormal pairing position (Fig. 15). There are 10 bivalents in both the plates; the XY-bivalent is seen in a central position in the plates

formation. In Figs. 12 and 13, a constriction is seen in the larger chromosome, which is supposedly a neo-X, and this constriction is about the length of the Y from the chiasma.

The X and Y seem always to pair normally although they have been seen separated at late diplotene (the stage of bouquet disintegration). However, even in the same individual and in the same cyst, the sex chromosomes at late diakinesis are seen closely connected in some of the cells, and separated by a considerable distance in others. When the connection is close and the mutual positions of the X and Y bivalent-like, there are two feebly stained Feulgen-positive strings to be seen between the two chromosomes. Sometimes there exists a loose connection seemingly maintained by only one such string, and quite often the X and Y merely lie side by side. Two "abnormal" situations, from adjacent cells, are seen in Figs. 14 and 15.

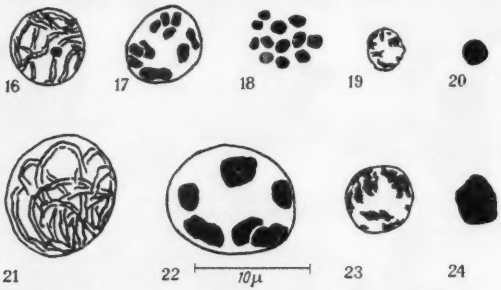
When the metaphase plate for the first division is formed, the X and the Y always (in the cells analyzed so far) mutually react as partners in a more or less normal co-orientation. It follows that the anaphase separation is normal, the sex chromosomes proceeding to opposite poles.

Probably the co-orientation is wholly normal after chiasmate connection has persisted since diplotene, and the abnormal-looking mutual positions of the sex chromosomes in the metaphase plate result from touch-and-go pairing succeeding a situation like that seen in Fig. 14.

It seems likely that one chiasma at least is always formed in the XY bivalent. When the chiasma terminalizes over the non-homologous end (where the formerly univalent X was once attached to what was then an autosome), the following late diakinesis and first metaphase show the abnormalities above described.

2. Cytophotometric observations

The large size of the chromosomes of *Xerophloea viridis* and other primitive Cicadellids (*Ulopa*, *Eupelix*, *Aphrodes*, *Strongylocephalus*; see



Figs. 16—24. *Graphocephala coccinea* (upper row) and *Xerophloea viridis*. Shown are stages used in the microphotometric (DNA-) measurements. In the upper row, from left to right: pachytene, diakinesis, first metaphase, early spermatid, late spermatid. In the lower row: pachytene, diakinesis, early spermatid, late spermatid. Not all the chromosomes are shown in the diakinesis nuclei

HALKKA 1959) as compared with other leafhoppers, indeed with any *Auchenorrhyncha*, suggested a relatively high DNA content. Therefore, Feulgen cytophotometry was applied to *Xerophloea* and to a common Cicadellid species with chromosomes of "normal" size, *Graphocephala coccinea*. The use of the two-wavelength method made possible the use of different types of nuclei.

In *G. coccinea*, nuclei in late pachytene, diakinese, first metaphase as well as early and late spermatid nuclei (Figs. 16—20) were measured, in *X. viridis* late pachytene, a few diakinese and early and late spermatid nuclei (Figs. 21—24). The result is given in Fig. 25. The histograms show that the amount of DNA in the pachytene, diakinesis and first metaphase nuclei of *G. coccinea* is about equal to the DNA content in the spermatids of *X. viridis*. When corresponding stages in *Xerophloea* and *Graphocephala* are compared, the amount of DNA in the latter is

found to be a little less than one-fourth of that in the former. Although no measurements on other leafhopper species have been made, it seems legitimate to infer that the DNA content of *Graphocephala* is not very far from the cicadellid average, while the amount of this substance in *Xerophloea* is exceptionally high, corresponding to the unusually large size of the chromosomes.

Moreover, since the measurements were made with the apparatus used by the SCHRADERS, a rough comparison with the *Pentatomidae (Heteroptera)* seems possible. HUGHES-SCHRADER and SCHRADER (1957)

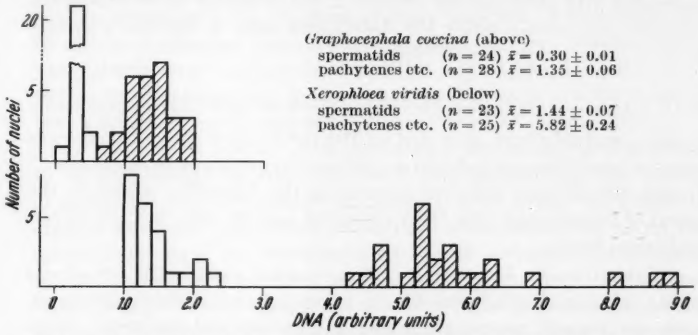


Fig. 25. DNA content of different types of nuclei of *Graphocephala coccinea* (above) and *Xerophloea viridis*. Results with spermatids are shown in white, results with pachytenes, diakineses and first metaphases in black. n = number of nuclei measured

used the one-wavelength method, measuring at the peak extinction wavelength. If this method is applied to the nuclei of *Xerophloea* at the late pachytene stage when the chromatin is most homogeneously dispersed, the extinctions obtained rise to values exceeding 0.9. To take a practical example, if the diameter of the nucleus is 10.3 μ, the diameter of the plug 6.5 μ, and the extinction 0.93, then the amount of DNA in arbitrary units is about 18.5. (Actually, it is not at all appropriate to use the one-wavelength method here.) The highest value obtained by the SCHRADERS (op. cit.) in the family *Pentatomidae* is 4.02 in *Acrosternum pennsylvanicum*. Although only a direct comparison can provide a definite answer, it may be tentatively suggested here that the nuclei of *Xerophloea* contain an amount of DNA much larger than that present in the *Pentatomidae* richest in this substance.

The nuclear volumes, at corresponding stages, of *Xerophloea* and *Graphocephala* are also different. At late pachytene, the volume of *Graphocephala* nuclei is about 220 μ^3, while that of *Xerophloea* nuclei is about 750 μ^3. (The maximum cross section areas of twenty nuclei

from each species were determined, and the volumes calculated on this basis.) The nuclear volumes thus stand in the ratio of 3.4 to 1. Thus, the volumes do not completely reflect the difference in the DNA values. To take a similar example from as close a taxonomic neighborhood as possible, the paper by HUGHES-SCHRADER (1957) may be cited.

Discussion

The above observations, diverse as they are, call for a discussion concentrating on three points: 1. Chromosome spiralization phenomena correlated with other nuclear events. 2. The behavior of the chiasmata and their significance within the *Auchenorrhyncha*. 3. Variable polyteny in the auchenorrhynchous *Homoptera*.

1. Chromosome spiralization phenomena correlated with other nuclear events

It is probably true, as stated by RIS (1957), that the "chromosomes reveal a whole hierarchy of helical systems superimposed on one another" (p. 55), but all that is known concerning this hierarchy relates to the spiral of lowest order (the DNA molecule) and the two or three spirals of highest order.

Without doubt, interesting coiling systems exist in the prophases of the primitive leafhoppers, but an attempt to relate these systems to each other would necessitate a specific experimental approach. However, even routine squashes such as are used in the present study, afford some possibilities for speculation. The number of bands at diplotene is approximately the same as the number of knotlike chromomeres at pachytene (Figs. 2, 8). These pachytene chromomeres are elongated in the direction of the axis of the chromosome. It may be assumed that it is precisely these chromomeres which are developed into bands later at diplotene. Some change possibly occurs in the mode of spiralization within the chromomere to make it shorten in the axial direction. Perhaps the change affects the submicroscopic units which compose each chromatid, rather than a bundle spiral formed by the units. In other words, the microscopic effect may be the sum of submicroscopic events.

At diplotene, the interband regions are either Feulgen-negative or very feebly Feulgen-positive without any observable longitudinal structures beyond the two chromatids. These, too, often fade out of sight. Where the structure has not been affected by differential stretching, the spiralization (banding) pattern is similar in the two chromosomes.

After diplotene, the chromatids shorten rapidly, and all banding pattern is soon abolished. This probably results from the formation of a microscopic coil, indications of which are seen in Fig. 13. After this stage, the chromosomes still shorten considerably, but the change

occurring in spiralization cannot be followed in ordinary squash preparations.

At diplotene, the chromosomes of *Xerophloea viridis* are remarkably like the primary polytene chromosomes seen in many tissues of young Dipteran larvae. However, in the leafhopper chromosomes spiralization is in process of increasing while, through endomitosis, spiralization in the chromosomes of the Dipteran larva is in process of decreasing. The external appearance of the chromosomes shown in Figs. 8 and 9 is not very different from the habitus of the primary polytene chromosomes in *Calliphora* (BIER 1957, Fig. 4 b). How far the mode of spiralization is homologous in Dipteran primary polytene chromosomes and in meiotic leafhopper chromosomes, remains obscure. However, it is likely that both are polytene and that this polytene condition influences the mode of spiralization in somewhat similar ways in both cases, to give a similar external appearance to both types of chromosomes.

In many plant families, it is not unusual for the degree of polyteny to be high enough to affect the mode (and perhaps also the timing) of spiralization in the meiotic prophase so as to render it possible to observe structural regularities which may reveal something about the genetic make-up of the chromosome. One such instance is the mapping of rye chromosomes by LIMA-DE-FARIA (1952), based on the analysis of the pachytene chromosomes of this cereal. In the animal kingdom, the meiotic prophase is seldom suitable for even modest mapping. Among the insects, some Orthopterans possess fairly large chromosomes, but the conditions of their meiotic prophase seldom encourage even limited attempts at mapping. Apparently the chromosomes of the primitive insect *Thermobia domestica (Thysanura)* are also very thick, as is seen in the pictures included in the paper by PERROT (1933). The present author's interest was drawn to this insect by Dr. S. R. BAWA, who has photographed the beautiful pachytene chromosome spirals in *Thermobia* in living condition (BAWA, in press).

In *Xerophloea*, it was not possible to establish whether the banding pattern is similar in homologous chromosome sections of different individuals, although this is to be expected. The definite disadvantage of the *Xerophloea* case is that diplotene is a very transient phase, and the number of nuclei with banded chromosomes obtained from any one individual is very limited. If this technical difficulty can be overcome and a comparison of banding patterns in two related primitive leafhoppers performed (there are about 13 *Xerophloea* species in the United States, for instance), the result will certainly be of interest, because leafhoppers possess diffuse centromeres.

It is noteworthy that the timing of spiralization is different in *Ulopa* and *Xerophloea*. Polarization phenomena as well as the behavior

of chiasmata seem to be quite independent of the spiralization cycle of the chromosomes in these insects. Strong persistence of the polarized condition is typical of all the primitive leafhoppers dealt with here.

2. The behavior of chiasmata and their significance in the Auchenorrhyncha

Among the *Auchenorrhyncha* there are only a few leafhopper species, such as *Eupelix* and *Xerophloea*, in which the chiasmata can be seen at approximately the loci where they originate. Even in these species, the fact that the bouquet orientation still persists when the chiasmata are first visible, complicates the analysis considerably. There is always enough interbivalent attraction to hold the bivalent ends together and to veil a considerable proportion of the chiasmata in the tangled maze of the bouquet. It is not impossible that chiasmata arise in bouquet loops in other cicadellids, too, but in most species the bivalents are much smaller than in the two mentioned above, and their component chromatids too delicate for analysis of chiasmata.

It is notable that only in the small and medium-sized bivalents of *X. viridis* are interstitial chiasmata seen at late diakinesis. It may be that in the large bivalents the chiasmata are to a lesser or greater extent terminally localized. However, while it is possible to establish that chiasmata are never present in the middle region of a large bivalent at diakinesis, it is not possible to obtain absolute proof that such chiasmata do not exist at the diplotene bouquet stage. Therefore the possibility is not excluded that terminalization takes place more rapidly in the long bivalents than in the short ones. This could be explained on the ground that there is more repulsive force per chiasma located in the long bivalents, i.e., the centromere being diffuse, the number of active repelling points is larger. This reasoning implies that the greater part of chiasma terminalization has already occurred before the chiasmata have become symmetrical. While it is quite unusual for this to happen, the bouquet-loop chiasmata of some *Auchenorrhyncha* are also somewhat unusual occurrences.

At the bouquet stage, associations between bivalent ends seem to be of universal occurrence in the *Auchenorrhyncha*. After the disintegration of the bouquet, the extent of these associations varies from species to species. In *Cicadula*, long chains of bivalents are seen at diakinesis (HALKKA 1959), in *Eupelix* the nonchiasmate ends of the two chromosomes of each rod bivalent are connected, and in *Xerophloea* practically all nonchiasmate bonds are abolished at or soon after bouquet disintegration.

The low frequency of chiasmata, together with the relatively small number of bivalents in species like *Eupelix* and *Xerophloea* adds up to quite a low recombination index. It seems probable that, in the

main, the number chromosomes has decreased in the group *Auchenorrhyncha* during its evolution so far (HALKKA 1959). Since in most cases no compensating increase in chiasma frequency has occurred (for instance, in *Limotettix* with only five autosome bivalents there is only one chiasma in each bivalent; HALKKA, op. cit.), it is concluded that the index of recombination is undergoing a downward trend in the group. This trend may have some advantageous evolutionary significance, since the genically controllable chiasmate recombination can now separate or keep together two genes which earlier belonged to different chromosomes and were subjected to the generally non-controllable Mendelian recombination. However, the decrease in recombination index has also almost certainly brought about some decrease in the flexibility of the evolutionary mechanism of the species. This may happen to species which become increasingly specialized, or in other words, inhabit a continuously narrowing niche, restricting themselves to a single food plant, for instance. So far, no attempts to correlate feeding habits with recombination index have been made, and if such work is ever done it must be quite extensive to have any statistical meaning.

The fact that in the leafhoppers polarization often transcends the limits between which it is normally confined, extending to diplotene, together with the varying intensity of the diffuse stage in the *Auchenorrhyncha*, makes it worthwhile to question whether chiasmata are of universal occurrence in the group. As a result of comparative studies on more than a hundred species belonging to different subgroups (HALKKA 1959), the present author believes that even in the *Fulgorina*, where the diffuse stage completely veils all pachytene and early diplotene events, chiasmata are responsible for maintaining connection between the bivalent halves. From easily analyzable cases like *X. viridis*, there is a complete series of decreasing observability to cases like *Calligypona*, where the bivalents emerge from the diffuse stage as practically fully formed dumb-bells.

3. Variable polyteny in the auchenorrhynchous Homoptera

It was stated by HALKKA (1959, p. 53) that "the principle of variable polyteny . . . seems to be only of secondary significance, if any, in the *Auchenorrhyncha*". The choice of words was somewhat incorrect, since what was meant was that no instances of doubling of chromosome number, accompanied by decrease in degree of polyteny, have been found in the group.

The species pair *Graphocephala coccinea* — *Xerophloea viridis* alone shows that differential polyteny exists in the *Auchenorrhyncha*. Moreover, since *X. viridis* is a representative of the primitive Cicadellids

(externally characterized by a flattened, elongated head with a sharp front edge), and *Graphocephala* is an "advanced" species, the difference in their DNA content is of great evolutionary significance. This conclusion obtains additional support from the fact that other primitive genera such as *Ulopa*, *Eupelix*, *Aphrodes* and *Strongylocephalus* are in possession of unusually large chromosomes. It seems improbable that all these primitive genera should have independently experienced an increase in their DNA content. It is likely that the large amount of DNA is just another primitive feature, which implies that in most lines of evolution in the *Cicadellidae* a decrease in the DNA content has taken place. The direction of the change — less DNA per Mendelian unit — would thus be the same as in *Thyanta* (SCHRADER and HUGHES-SCHRADER 1956), diaspine coccids (HUGHES-SCHRADER 1957) and *Banasa* (SCHRADER and HUGHES-SCHRADER 1958).

Many of the primitive species which, according to the present author's hypothesis, have retained the ancestral DNA content are quite common and are what may be defined as successful species. Thus, if the hypothesis is true, it remains to be explained what kind of evolutionary advantage the advanced species have gained through decrease in the degree of polyteny. Perhaps the degree of polyteny is of some significance in what is called the "mutability" of a species, in the sense that thinner chromosomes may be more easily breakable and mutable than thicker ones. The primitive genera of the *Auchenorrhyncha* are often represented by one or a few species only in a geographic region of considerable size.

The details of the actual event of decrease in polyteny cannot but remain obscure. The fact that *G. coccinea* is two steps below *X. viridis* in DNA content suggests that in cicadellids related to both *Graphocephala* and *Xerophloea* the intermediate level may be represented. If so, the situation would only parallel that apparently prevailing in the Heteropteran family *Pentatomidae*, in which three DNA levels have been revealed (HUGHES-SCHRADER and SCHRADER 1956).

The SCHRADERS (1958, p. 209) anticipated electron microscopy would be needed to provide direct evidence of the validity of the differential polyteny hypothesis. Happily, the peculiar conditions prevailing in the meiotic prophase of primitive leafhoppers seem to provide this proof at light microscope level. It is true that the number of ultimate subunits in the chromosomes of *Xerophloea* and *Graphocephala* cannot be ascertained, but the chromosomes of the former, as stained with Feulgen, are distinctly thicker than those of the latter. At every stage of meiosis, the chromosomes of *X. viridis* are not only thicker but also longer than those of *G. coccinea*. While the difference in length is attributable to the mode of spiralization, the latter, in turn, probably is influenced by the degree of polyteny.

It may be too naive to assume that, within the *Cicadellidae*, the sharper the front edge of the head, the larger the chromosomes. However, a search among the relatives of *Xerophloea viridis* would perhaps reveal even larger chromosomes than those found in this species. In addition to *Ledrinae*, *Ulopinae* and *Eupelicinae*, the list of hopeful subfamilies includes at least the *Dorycephalinae* and the *Hecalinae*. All these subfamilies are placed close to the base of the family tree of the *Cicadellidae* by most expert taxonomists. Within the family *Cicadellidae*, the size of the chromosomes varies widely from species to species in the more than 90 representatives so far studied cytologically. The existence of differential polyteny in the group *Auchenorrhyncha* is welcomed, as in so many cytological respects the group is almost monotonously uniform, in sharp contrast to the next neighbor, the *Sternorrhyncha*, with its well-known "cockeyed coccids".

Summary

1. The chromosomes of the cicadellid species *Xerophloea viridis* ($2n = 18 + XY$) and of species belonging to related genera, are far larger than the chromosomes of any other species belonging to the *Auchenorrhyncha* so far cytologically studied.

2. In *X. viridis*, at diplotene (and in *Ulopa reticulata*, a related species, at pachytene), the chromosomes of the bivalents show a banded appearance somewhat like that seen in the primary polytene chromosomes in the young larvae of many Dipteran species. It is suggested that a relatively high degree of polyteny is responsible for the peculiar spiralization patterns in the meiotic prophase of *X. viridis*.

3. In *X. viridis* (and in *Eupelix cuspidata*, a related species) the chiasmata arise at a stage when the bouquet orientation still persists.

4. The chiasma frequency is low in the *Auchenorrhyncha*. In many cases, a lowering of the recombination index has taken place during the chromosomal evolution of a genus or tribe. It is suggested that chiasmata are of universal occurrence in the *Auchenorrhyncha*.

5. The amount of DNA contained in the nuclei of *Graphocephala coccinea* ($2n = 22 + X$), another member of the family *Cicadellidae*, is about one-fourth of that contained in the nuclei of *Xerophloea viridis* ($2n = 18 + XY$) at the same stages.

6. Probably *Xerophloea* and other primitive genera with large chromosomes have retained the ancestral amount of DNA, typical of the family *Cicadellidae* at the time when more advanced tribes began to develop. This implies that in the line leading to *Graphocephala* two downward shifts in the DNA content, and in the level of polyteny, have occurred.

References

ANSLEY, H. R.: A cytological and cytophotometric study of alternative pathways of meiosis in the house centipede (*Scutigera forceps* RAFINESQUE). Chromosoma (Berl.) **6**, 656—695 (1954).

BEIRNE, B. P.: Leafhoppers *(Homoptera Cicadellidae)* of Canada and Alaska. Canad. Entomol. **88**, Suppl. 2, 1—180 (1956).

BIER, K.: Endomitose und Polytänie in den Nährzellkernen von *Calliphora erythrocephala* MEIGEN. Chromosoma (Berl.) **8**, 493—522 (1957).

BORING, A. M.: A study of the spermatogenesis of 22 species of the *Membracidae, Jassidae, Cercopidae* and *Fulgoridae* with especial reference to the behavior of the odd chromosome. J. exp. Zool. **4**, 469—514 (1907).

GEITLER, L.: Endomitose und endomitotische Polyploidisierung. Protoplasmatologia **94**, 1—89 (1953).

HALKKA, O.: Studies on the chromosomes of the *Hemiptera, Homoptera, Auchenorrhyncha*. Ann. Acad. Sci. fenn. A, IV **43**, 1—72 (1959).

HUGHES-SCHRADER, S.: Differential polyteny and polyploidy in diaspine coccids. Chromosoma (Berl.) **8**, 709—718 (1957).

—, and F. SCHRADER: Polyteny as a factor in the chromosomal evolution of the *Pentatomini (Hemiptera)*. Chromosoma (Berl.) **8**, 135—151 (1956). — The *Nezara* complex *(Pentatomidae-Heteroptera)* and its taxonomic and cytological status. J. Morph. **101**, 1—24 (1957).

LEUCHTENBERGER, C.: DNA-determination by microspectrophotometry. Gen. cytochem. Methods **1**, 219—278 (1958).

LIMA-DE-FARIA, A.: Chromomere analysis of the chromosome complement of rye. Chromosoma (Berl.) **5**, 1—68 (1952).

OMAN, P. W.: The nearctic leafhoppers *(Homoptera: Cicadellidae)*. Mem. entom. Soc. Wash. **3**, 1—253 (1949).

PERROT, J. L.: La spermatogenèse et l'ovogenèse de *Lepisma (Thermobia) domestica*. Hétéropycnose dans un sexe homogamétique. Z. Zellforsch. **18**, 573—592 (1933).

RIS, H.: Chromosome structure. Symposia of the McCollum-Pratt Institute. A symposium on the chemical basis of heredity. 1957, pp. 23—69.

SCHRADER, F., and S. HUGHES-SCHRADER: Polyploidy and fragmentation in the chromosomal evolution of various species of *Thyanta (Hemiptera)*. Chromosoma (Berl.) **7**, 469—496 (1956). — Chromatid autonomy in *Banasa (Hemiptera: Pentatomidae)*. Chromosoma (Berl.) **9**, 193—215 (1958).

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DER NUKLEOLUS ALS LEBENSWICHTIGER BESTANDTEIL DES ZELLKERNES*

Von

Wolfgang Beermann

Mit 11 Textabbildungen

(Eingegangen am 26. Februar 1960)

A. Einleitung

Die Beobachtungen von Heitz (1931a, b) und die cytogenetischen Experimente von McClintock (1934) haben erwiesen, daß der Nukleolus als deutlich abgegrenzter und gesetzmäßiger Bestandteil fast aller Typen von Zellkernen in seiner Entstehung nicht autonom ist. Nukleolen entstehen im Zusammenhang mit genetisch festliegenden Chromosomenabschnitten, den Nukleolen-Bildungsorten, denen sie im Interphasekern gewöhnlich fest anhaften. McClintock hat diesen Sachverhalt auf der Grundlage ihrer eigenen Befunde und der Beobachtungen von Heitz und von Navashin (vgl. McClintock 1934, S. 326, Addendum) in bestimmter Form interpretiert. Nach ihren Vorstellungen wird die Nukleolensubstanz nicht von den Bildungsorten selbst produziert, sondern entsteht gewissermaßen als gemeinsames Stoffwechselprodukt aller Chromosomen, um dann von den Nukleolenbildungsorten als den „organizers" gesammelt und zu einheitlichen Körpern, den Nukleolen, vereinigt zu werden.

Die Hypothese von McClintock gründet sich im besonderen darauf, daß Kerne, die sicher keine Nukleolenbildungsorte besitzen, doch „Nukleolen" enthalten können: So entwickeln nach den Befunden von Creighton (vgl. McClintock l. c.) Mikrosporen vom Mais, denen infolge eines induzierten Stückverlustes im Chromosom 6 der „organizer" fehlt, zwar keinen einheitlichen Nukleolus, weisen aber nukleolenartiges Material auf, das den Chromosomen in Tropfen anhaftet. Heitz (1931b) und andere Autoren haben ferner beobachtet, daß Teilkerne (Karyomeren), wie sie nach zufälliger Absprengung einzelner Chromosomen in der Mitose oder gesetzmäßig in der Furchung von Copepoden entstehen, „Nukleolen" besitzen, auch wenn das Chromosom, aus welchem der Teilkern gebildet wird, zum Nukleolus normalerweise keine Beziehung erkennen läßt. Es scheint also, wie Bauer (1935, 1936) nach Untersuchung

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der Nukleolenverhältnisse in Chironomiden-Speicheldrüsenkernen vermutet, daß die Funktionen der normalen Nukleolenbildungsorte bei Bedarf auch von anderen Chromosomenabschnitten übernommen werden können, oder aber, daß die Nukleolensubstanz die Fähigkeit besitzt, sich auch selbständig aus dem Kernsaft abzuscheiden. Diese Vermutungen sind zwar vom rein morphologischen Standpunkt aus nicht unberechtigt, wenn man „Nukleolus" stets gleich „Nukleolus" setzt, doch fehlt ihnen eine sichere chemische und experimentelle Grundlage.

Physiologische Untersuchungen zum Problem der Nukleolen haben sich meist darauf beschränkt, Zusammenhänge zwischen Größe, Struktur oder Chemie des Nukleolus einerseits und der Zellfunktion andererseits zu ermitteln. Solche Untersuchungen sagen über die Lebenswichtigkeit der Nukleolenbildungsorte und der Nukleolen wenig aus. Man wird auf das Experiment von CREIGHTON und MCCLINTOCK zurückgreifen müssen, um die Entbehrlichkeit der Bildungsorte, nicht bloß für die Nukleolenbildung an sich, sondern für die Funktion von Kern und Zelle überhaupt zu prüfen. Über die Lebensfähigkeit oder über Fehlleistungen von „nukleolenlosen" Zellen (d. h. solchen, denen die Nukleolenbildungsorte fehlen) haben die genannten Autorinnen und spätere Untersucher nicht berichtet, aus Gründen, die in der Natur des Experimentes liegen: Mikrosporen mit defizienten Chromosomen sind nicht entwicklungsfähig. Bei Tieren fällt zwar die gametophytische Sterilitätsschranke weg, doch bleibt die theoretische und praktische Schwierigkeit bestehen, die Effekte des Verlustes von Nukleolenbildungsorten von der Wirkung der gleichzeitig induzierten Chromosomen-Stückverluste zu trennen. Diese Schwierigkeit läßt sich unter bestimmten Voraussetzungen umgehen: Die cytogenetische Situation in der Untergattung *Camptochironomus* der Familie *Chironomidae (Diptera, Nematocera)* erlaubt es, Zygoten ohne Nukleolenbildungsorte durch Rekombination, also ohne die Induktion von Chromosomen-Stückverlusten, herauszuzüchten. Sie gestattet es ferner, verschiedene und verschiedenartige Nukleolenbildungsorte für sich oder zusammen auf ihre Leistungsfähigkeit zu untersuchen. Eine vorläufige Mitteilung über die Ergebnisse ist erschienen (BEERMANN 1959).

B. Material und Methoden

Die Versuchstiere sind *Chironomus tentans*, *C. pallidivittatus* und Tiere aus Nachzuchten der Bastarde der beiden Arten. Näheres über Aufzucht, Haltung und Kreuzungsmethoden findet sich in früheren Arbeiten (BEERMANN 1952, 1955). Weitere Einzelheiten, insbesondere zur cytologischen Methodik, werden unter den entsprechenden Befunden beschrieben.

C. Ergebnisse

1. Die Nukleolenbildungsorte und Nukleolen von C. tentans, C. pallidivittatus und den Bastarden

Die Nukleolenverhältnisse bei den untersuchten Arten und den Bastarden sind in anderem Zusammenhang (BEERMANN 1952, 1953, 1955) bereits beschrieben worden. Die Ergebnisse werden hier noch einmal zusammengestellt und ergänzt. Zunächst eine Bemerkung zur Terminologie:

BAUER (1935) unterschied in den Chironomiden-Speicheldrüsen Hauptnukleolen von Nebennukleolen. Erstere sind die regelmäßig gebildeten normalen Nukleolen, während als Nebennukleolen die Tröpfchen bezeichnet werden, die sich unter bestimmten Bedingungen an einzelnen Chromosomenorten abscheiden. Nach der einleitend gegebenen Charakteristik des Nukleolus sind aber die Nebennukleolen keine echten Nukleolen, denn die Chromosomenorte, an welchen sie entstehen, sind solche, deren Aktivität in engem Zusammenhang mit dem Funktions- bzw. Differenzierungszustand der Zelle variiert (BEERMANN 1952). Als „Bildungsorte" der Neben-„Nukleolen" fungieren also in verschiedenen Zellen ganz verschiedene Chromosomenorte. „Echte" Nukleolenbildungsorte sind dadurch definiert, daß sie unabhängig vom Zelltyp und von der Zellfunktion stets Nukleolen bilden (von bestimmten Ausnahmen abgesehen, vgl. Diskussion).

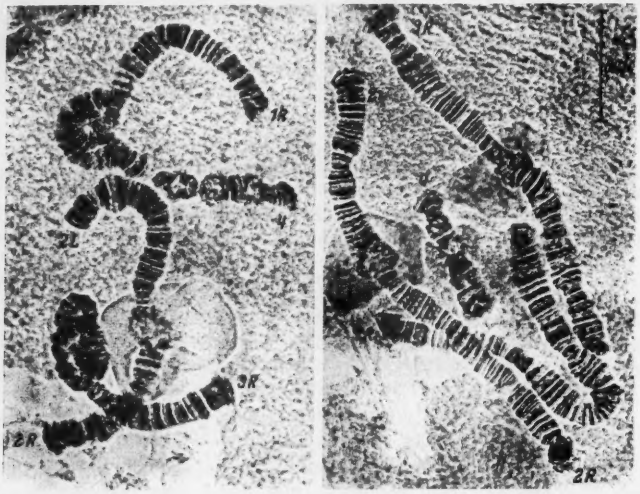
In den untersuchten europäischen Populationen (Norddeutschland, Österreich, Schweden, England) besitzt *Chironomus tentans* 2 Nukleolenbildungsorte, einen im Chromosomenarm 2 L und einen im Chromosomenarm 3 L (Abb. 1). Dazu kommt — homozygot oder heterozygot — in manchen Individuen ein „akzessorischer" Nukleolenbildungsort[1] terminal am Chromosom 4 (s. S. 282). Der Nukleolenbildungsort in 3 L fehlt in den meisten *tentans*-Populationen in Nordamerika, die von ACTON (1958, 1959) untersucht wurden; in der Population „Churchill" ist er stets an die Inversion „3 Lc" (nach ACTONS Bezeichnungsweise) gebunden. Außerdem tritt in den kanadischen Populationen ein Nukleolenbildungsort im Chromosom 1 auf. Die normalen Nukleolenbildungsorte von *C. tentans* sind stets homozygot.

Den zwei normalen Nukleolenbildungsorten von *C. tentans* steht in den europäischen Populationen von *C. pallidivittatus* nur ein Nukleolenbildungsort im Chromosom 2 R gegenüber (Abb. 1). Er ist stets homozygot. *C. pallidivittatus* weist in manchen Bastardnachzuchten einen akzessorischen Nukleolenbildungsort auf, der wie bei *C. tentans* ganz am Ende des 4. Chromosoms liegt.

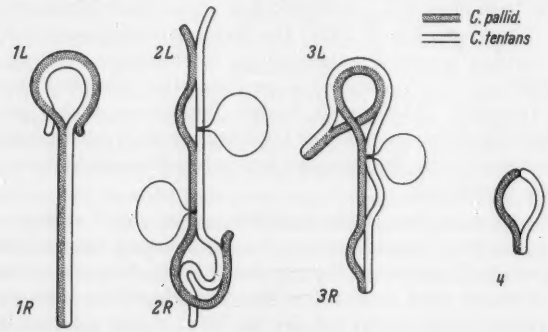
Die normalen Nukleolenbildungsorte von *C. tentans* und *C. pallidivittatus* liegen in Chromosomenabschnitten, die ihrer Organisation

[1] Ein in den Populationen sporadisch auftretender Nukleolenbildungsort von variabler Aktivität die an ihm entstehenden Nukleolen sind physiologisch den normalen Nukleolen nicht gleichwertig (S. 283) und werden als „Sondernukleolen" bezeichnet.

nach als „euchromatisch" gelten. Diese Bereiche sind in den Riesenchromosomen eindeutig in Scheiben und Zwischenscheiben gegliedert. Die Nukleolenbildungsorte selber erscheinen als „Querscheiben", deren



a



b

Abb. 1a u. b. Speicheldrüsenchromosomen und Nukleolen von (links oben) *Chironomus pallidivittatus*, rechts oben *Chironomus tentans*, und (unten, schematisch) vom Bastard der beiden Arten. Nach Orcein- bzw. Karmin-Essigsäure-Präparaten, Phasenkontrast. Vergr. (der Photographien) 350 ×

Struktur sich allerdings von der Struktur gewöhnlicher Querscheiben merklich unterscheidet (vgl. BEERMANN 1952, 1955, sowie Abb. 2). Sie sind nur schwach feulgenpositiv, scheinen aber proteinreich zu sein, denn

ihre Dichte ist nach elektronenoptischen Befunden (BEERMANN und BAHR 1954, Fig. 4) und nach der Darstellbarkeit im Phasenkontrast fast so hoch wie die der gewöhnlichen Querscheiben. Im Elektronenmikroskop erweist sich die Feinstruktur als im Gegensatz zu anderen Chromosomenabschnitten recht homogen. Lichtmikroskopisch und elektronenoptisch fällt die scharfe Abgrenzung der Nukleolenbildungsorte gegen die benachbarten Querscheiben auf, die im Gegensatz zu dem normalen „puffing" auch bei Verquellung und Vakuolisierung erhalten

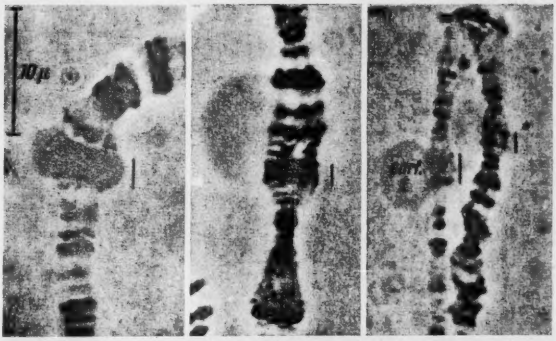


Abb. 2. Die Struktur von Nukleolenbildungsorten in Malpighigefäß-Chromosomen. Nach Orcein-Milchsäure-Präparaten, Phasenkontrast. Links: Bildungsort in 3L von *C. tentans*, junge Puppe. Mitte: Bildungsort in 2R von *C. pallidivittatus*, junge Imago. Rechts: *C. pallidivittatus*, 2R, mit einem vollständigen und einem Partialbildungsort (part. I, vgl. Text); letzterer gedehnt, daher länger als der normale Bildungsort. 2000 ×

bleibt. Es hat deshalb den Anschein, als ob der Nukleolenbildungsort auf beiden Seiten in einer scharf definierten Querscheibe endete; das kann aber auch Täuschung sein, denn röntgeninduzierte Fragmentationen der Nukleolenbildungsorte ändern das Bild nicht (vgl. S. 283 und Abb. 2 rechts). Verquellung, Vakuolisierung oder Aufsplitterung der Nukleolenbildungsorte werden im Zusammenhang mit verstärkter Aktivität beobachtet (also im Zusammenhang mit rascher Produktion von Nukleolensubstanz). Wenn z. B. die Nukleolen von Malpighigefäßkernen nach der Metamorphose mit zunehmendem Alter von Puppe und Imago immer kleiner werden, so bedeutet das, daß die Aktivität der Nukleolenbildungsorte langsam zum Stillstand kommt; und parallel dazu wird der Grad der Vakuolisierung bzw. Aufsplitterung der Bildungsorte immer geringer, bis sie in der Imago schließlich als Querscheiben (Doppelscheiben) von etwa 1,5 μ Dicke erscheinen. Ausnahmsweise kann in den Speicheldrüsen die Aufspaltung der Bildungsorte so weit gehen, daß ein Balbiani-Ring entsteht (BEERMANN 1952, Abb. 34). Dies wurde bisher zweimal in Laboratoriumszuchten beobachtet. In dem einen Fall hatte sich

der Nukleolenbildungsort im Chromosom 3L von *C. tentans* zu einem Balbani-Ring entwickelt, und zwar bei sämtlichen untersuchten Tieren einer Nachkommenschaft. In dem anderen Fall war der Bildungsort im Chromosom 2R von *C. pallidivittatus* betroffen, wiederum bei allen Tieren einer Nachkommenschaft. In beiden Fällen läßt sich die Möglichkeit nicht ausschließen, daß eine benachbarte Querscheibe, also nicht der Nukleolenbildungsort selbst, den Balbiani-Ring gebildet hatte. Die normale „Funktionsstruktur" der Nukleolenbildungsorte ist jedenfalls sicherlich nicht die des Balbiani-Ringes oder des „puff".

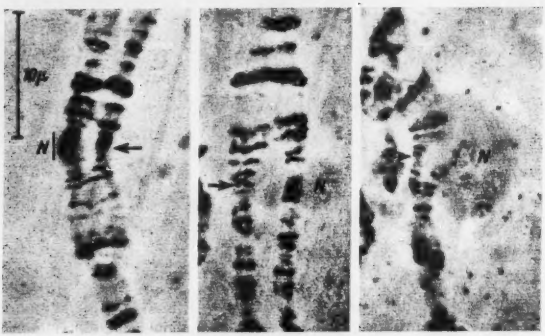


Abb. 3. Heterozygoter Nukleolenbildungsort in hybridem Chromosom 2R. Links und Mitte: Malpighigefäße von Vorpuppen; rechts: Rectum von Vorpuppe. Orcein-Milchsäure, Phasenkontrast. Die Stelle im *tentans*-Partner, an welcher im *pallidivittatus*-Partner der Nukleolenbildungsort eingefügt ist, wird durch einen Pfeil markiert. 2000 ×

Daß die Nukleolenbildungsorte von *C. tentans* und *C. pallidivittatus* sozusagen als zusätzliche genetische Strukturelemente in das normale Querscheibenmuster eingeschaltet sind, und zwar bei *C. pallidivittatus* an anderer Stelle als bei *C. tentans*, wird aus dem cytologischen Vergleich der Chromosomen der beiden Arten gefolgert: Im Bastard sind alle drei Bildungsorte, also der Bildungsort von *C. pallidivittatus* in Chromosom 2R ebenso wie die beiden Bildungsorte von *C. tentans* in 2L und 3L, heterozygot (vgl. Abb. 1, unten), liegen aber in Bereichen, deren Querscheibenmuster in beiden Partnern (also in beiden Species) bis auf die letzte erkennbare Scheibe das gleiche zu sein scheint (Abb. 3). Natürlich läßt es sich nicht mit Sicherheit entscheiden, ob dem Bildungsort des einen Partners nicht doch eine sehr feine Querscheibe im anderen gegenübersteht, doch grundsätzlich ist der Schluß berechtigt: Die Nukleolenbildungsorte der beiden Species liegen an einander nicht entsprechenden, nicht homologen Orten. Wie derartige Verschiedenheiten zustande kommen, ist eine Frage für sich; jedenfalls sind gewöhnliche Chromosomenmutationen als Ursache ausgeschlossen.

Der Bildungsort des akzessorischen oder „Sonder"-Nukleolus am Chromosom 4 von *C. tentans* liegt im Bereich der terminalen Querscheibe des 4. Chromosoms (Abb. 4), die wie alle „Telomeren" heterochromatisch ist. Es ist nicht zu entscheiden, ob der Sondernukleolus vor oder innerhalb des Telomers gebildet wird, oder ob er wirklich terminal entsteht. Für die letzte Möglichkeit sprechen Präparate, in denen das Telomer dem Nukleolus außen aufsitzt (Abb. 4, links), ohne in ihn einzudringen; solchen Bildern stehen aber andere gegenüber (Abb. 4, rechts), wo der ganze Sondernukleolus von fein aufgesplitterten Chromatinfäden durchzogen wird.

Die Form des Nukleolus und die Konstitution der Nukleolensubstanz sind bei den normalen Nukleolen und den Sondernukleolen verschieden. Der normale Nukleolus besitzt unregelmäßige Konturen, auch im ungequetschten lebenden Zellkern; seine Konsistenz ist zähflüssig.

Die Binnenstruktur der normalen Nukleolen wechselt. Eine durchgehende Granulation der Nukleolensubstanz, mit starker Auflockerung der Peripherie des Nukleolus, ist anscheinend für Phasen verstärkter Aktivität des Zellkernes charakteristisch (vgl. Abb. 5: „N2R"). Sonst zeigen die normalen Nukleolen glatte Konturen, eine granuläre Rindenschicht, und im Innern eine oder mehrere große Vakuolen, in welche wieder Kugeln der granulären „Rindensubstanz" eingelagert sein können. Die Sondernukleolen sind stets kugelrund. Auch sie lassen eine sehr feine Rindenschicht erkennen (Abb. 5). Im Innern zeigen sich zuweilen kleine Vakuolen. Normale Nukleolen und Sondernukleolen verschmelzen bei Berührung nicht miteinander (Abb. 5, rechts).

2. Die Züchtung von nukleolenlosen Zygoten und balancierten nukleolenheterozygoten Stämmen

Aus der Situation in den Artbastarden *C. tentans* × *C. pallidivittatus* folgt, daß bei weiterer Auskreuzung der Bastarde in der F_2 (und in den späteren Generationen) Zygoten mit allen Kombinationen von Nukleolenbildungsorten, unter anderem auch solche ohne jeden Nukleolenbildungsort, auftreten müssen. Bei näherungsweise freier Rekombination zwischen den Chromosomenarmen 2L und 2R im Bastard (etwa 35% im ♀ und 45% im ♂, vgl. BEERMANN 1953) sollte man annähernd $^1/_{64}$ solcher nukleolenloser (N^-) Zygoten pro F_2-Gelege erwarten. Der Erwartungswert ist zu gering, um mit Sicherheit den Nachweis zu führen, daß N^--Zygoten tatsächlich vorkommen, und um zu prüfen, welches Schicksal sie in der Entwicklung erleiden. Es wurde deshalb angestrebt, die Nukleolenbildungsorte durch planmäßige Züchtung schrittweise zu eliminieren, so daß im letzten Kreuzungsschritt die Eltern jeweils nur noch einen Nukleolenbildungsort heterozygot besaßen, also einfache „Nukleolen-Heterozygote" darstellten. Bei einer derartigen Kreuzung

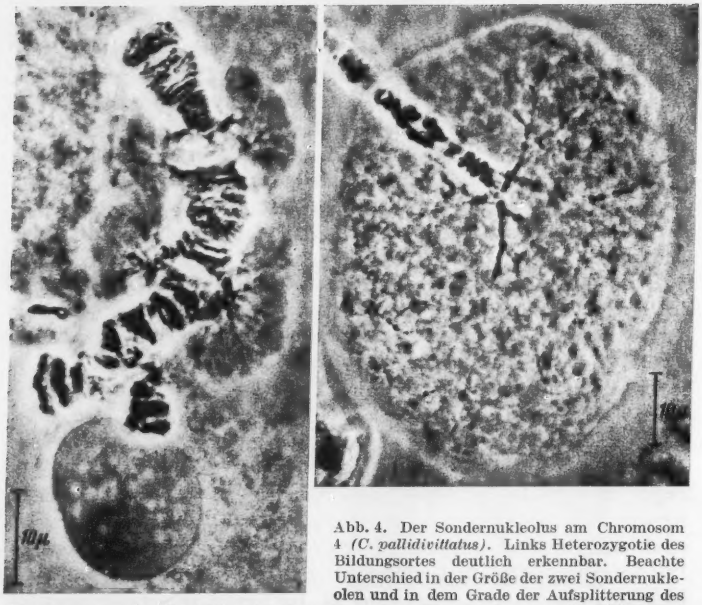


Abb. 4. Der Sondernukleolus am Chromosom 4 *(C. pallidivittatus)*. Links Heterozygotie des Bildungsortes deutlich erkennbar. Beachte Unterschied in der Größe der zwei Sondernukleolen und in dem Grade der Aufsplitterung des Chromosomenendes. Das Bild rechts stammt von einem Tier, das den normalen Bildungsort in 2R nur heterozygot besaß. Karmin-Essigsäure. Phasenkontrast. Links 1520 ×, rechts 1140 ×

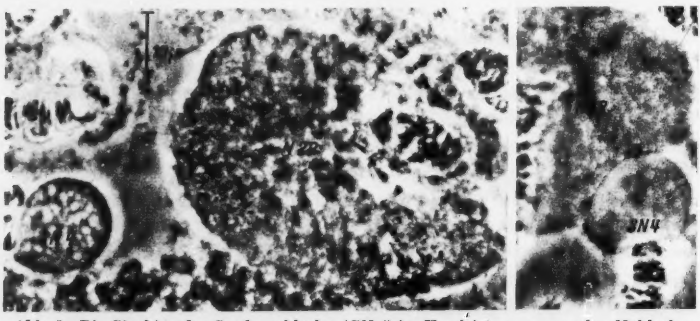


Abb. 5. Die Struktur des Sondernukleolus (*SN 4*) im Vergleich zum normalen Nukleolus (*N 2R*). Rechts: Der Sondernukleolus verschmilzt nicht mit dem normalen, trotz engem Kontakt. Karmin-Essigsäure, Phasenkontrast. 1260 ×

müßte dann $^1/_4$ aller Zygoten ohne Nukleolenbildungsorte bleiben. Im übrigen müßte es sich im Lauf der Kreuzungsarbeit auch herausstellen, ob die 3 verschiedenen Bildungsorte der Hauptnukleolen von *C. tentans*

+*C. pallidivittatus* einander vollständig vertreten können, also physiologisch gleichwertig sind oder nicht.

Das Ausgangsmaterial für die Heranzucht der nukleolen-heterozygoten Stämme bildete die F_9 eines Artbastardes aus dem Jahre 1954 (B VIII, vgl. BEERMANN 1955); die Linie war die ganze Zeit unkontrolliert in Bruder-Schwester-Inzucht weitergeführt worden, erwies sich aber noch als hybrid in allen 4 Chromosomen. Die F_9-Imagines wurden paarweise einzeln angesetzt und nach Ablage der Eier durch Analyse ihrer Malpighigefäß-Chromosomen auf ihre Chromosomen- und damit Nukleolenkonstitution geprüft. Unter 8 kontrollierten Paaren war ein geeignetes, das folgende Konstitution aufwies (t = *tentans*, p = *pallidivittatus*, N = Nukleolenbildungsort; Chromosom 1 R ist nicht bestimmbar, weil es in beiden Species gleich aussieht; Chromosom 4 bleibt oft unbestimmt auf Grund seiner geringen Größe):

	1L	1R	2L	2R	3L	3R	4
♀	t/p	?	pp	t/pN	tN/p	t/p	?
♂	tt	?	pp	t/pN	tN/p	t/p	?

Zu erwarten waren danach $^1/_{16}$ N^--Zygoten. Das Gelege („$F_{10/1}$") enthielt 864 befruchtete Eier (neben 158 unbefruchteten, d. h. solchen, die jede Art von Entwicklung vermissen lassen und darin vollständig denen gleichen, die von unbegatteten ♀♀ abgelegt werden, vgl. Abb. 6, oben rechts). Es schlüpften 774 Larven, 37 weitere Embryonen hatten sich normal entwickelt, die Larven schlüpften aber nicht; dagegen zeigten die übrigen 53 Embryonen, also fast genau $^1/_{16}$ von 864, charakteristische Entwicklungsstörungen. Es hat sich herausgestellt, daß dieser Typus von Letalembryonen die N-Zygoten repräsentiert (Abb. 6). Die Art der Fehlentwicklung wird später kurz skizziert und besprochen. Hier sei nur hervorgehoben, daß N^--Letalembryonen äußerlich (bei der Inspektion der Eier unter dem Binokular) bereits so charakteristische Merkmale aufweisen, daß eine Verwechslung mit anderen Letalzygoten kaum vorkommt. Das ist zur Beurteilung der später aufgeführten weiteren Spaltungsergebnisse wichtig (vereinzelte unsichere Eigelege wurden verworfen).

Die aus dem Gelege „$F_{10/1}$" geschlüpften Larven wuchsen zu Imagines heran, die paarweise angesetzt und nach der Ablage der Eipakete wiederum auf ihren Chromosomenbestand geprüft wurden. Von 61 geschlüpften Imagines, darunter 21 ♀♀, glichen 45 in ihrer Nukleolenkonstitution ihren Eltern, besaßen also je einen heterozygoten Nukleolenbildungsort im Chromosomenarm 2 R (*pallidivittatus* zugehörig) und im Chromosom 3 (*tentans* zugehörig); 8 Tiere besaßen nur einen einzelnen heterozygoten Nukleolus im Chromosomenarm 2 R, 7 Tiere nur einen einzelnen heterozygoten Nukleolus im Chromosom 3. Ein weiteres

Tier gehörte der Klasse „2L N^-N^-; 3 NN“ (Nukleolus im 3. Chromosom homozygot, sonst keine Nukleolen) an. Die übrigen 5 Rekombinationsklassen (darunter selbstverständlich die N^--Zygoten) waren nicht vertreten. Die starke Abweichung vom theoretischen Spaltungsverhältnis (1:2:1:2:4:2:1:2:1), die in der Begünstigung der doppelt Heterozygoten zum Ausdruck kommt, braucht mit der Nukleolensituation nichts zu tun zu haben; sie bestätigt nur andere eigene Erfahrungen über Heterosiseffekte bei Bastardnachkommenschaften (vgl. auch die weiteren Spaltungsergebnisse sowie die oben angeführte Tatsache, daß trotz

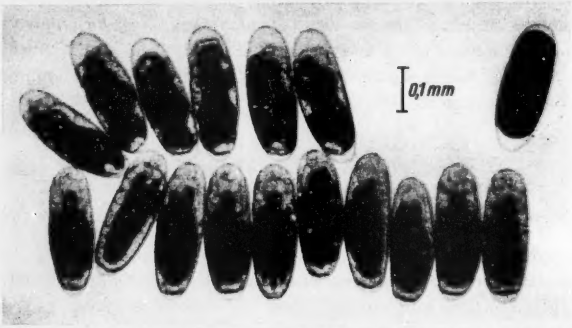


Abb. 6. Embryonen aus einer Kreuzung von Nukleolenheterozygoten, Alter (20° C) etwa 24 Std.; Vorderenden nach oben. Untere Reihe: 10 normale Embryonen; Zweites Ei von links in Seitenansicht; sonst von dorsal gesehen. Oben: 6 nukleolenlose (N^-) Embryonen. Rechts oben ein unbefruchtetes Ei. Lebend, 70 ×

Bruder-Schwester-Inzucht über 9 Generationen keine Homozygotisierung eingetreten war).

Aus dem Auftreten vitaler und fertiler Imagines mit nur einem einzigen Nukleolenbildungsort ergibt sich, daß die in dieser Kreuzung erfaßten zwei Nukleolenbildungsorte jeder für sich, und schon in einfacher Dosis, normale Vitalität und Fertilität gewährleisten. Letal sind nur diejenigen Zygoten, die überhaupt keinen Nukleolenbildungsort besitzen. Unter 15 „F_{11}“-Gelegen, d. h. den Nachkommenschaften der „$F_{10/1}$“-Imagines, war eines, dessen Eltern beide nur einen einzigen, und zwar den gleichen, heterozygoten Nukleolenbildungsort besaßen („$F_{11/18}$“). Die Chromosomenkonstitution der Eltern war:

	1L	2L	2R	3L	3R	4
♀	t/p	pp	tt	tN/p	t/p	?
♂	t/p	pp	tt	tN/p	t/p	?

Die Nachkommen sollten also jetzt zu $^1/_4$ aus letalen N^--Zygoten bestehen. Das nachzuprüfen, war auf Grund der allgemein hohen embryo-

nalen Letalität in der vorliegenden Kreuzung nicht möglich. Es wuchsen nur 12 Imagines, darunter 4 ♀♀, auf, die sämtlich wieder Nukleolenheterozygote waren. Die Weiterzucht der Linie mißlang: Alle 4 ♀♀ erwiesen sich als ganz oder nahezu steril. Die Ursache ist auch hier eher in der übrigen genetischen Konstitution (ungünstige Kombination von *tentans*- und *pallidivittatus*-Chromosomen, Homozygotisierung) als in der Tatsache zu suchen, daß die Tiere nur noch einen einzigen heterozygoten Nukleolus besaßen.

Die Zuchtschwierigkeit konnte dadurch umgangen werden, daß eines der ♂♂ aus der Zucht „$F_{11/18}$" mit einem normalen *pallidivittatus*-♀ rückgekreuzt wurde. Diese Kreuzung war voll fertil und ließ die Aufzucht von zahlreichen Imagines („$F_{11}R$") zu. Die Rückkreuzung mit *pallidivittatus* läßt sich wie folgt symbolisieren:

pall. 2R NN; $3N^-N^- \times$ „F_{11}" 2R N^-N^-; $3N/N^-$.

Man erkennt, daß alle Nachkommen heterozygot in 2R, nämlich N/N^- (p/t), sein müssen, und daß 50% der Nachkommen außerdem im 3. Chromosom keinen Nukleolenbildungsort besitzen werden. Damit war die Schaffung eines neuen Stammes von Nukleolenheterozygoten, diesmal auf der Basis einer Hybridität im Chromosomenarm 2R, möglich geworden und außerdem der übrige Chromosomensatz weitgehend dem reinen *pallidivittatus*-Bestand angenähert, was zur Stabilisierung von Vitalität und Fertilität erstrebenswert ist.

Die Imagines von „$F_{11}R$" lieferten 14 verschiedene, in Bezug auf die Spaltungsverhältnisse, die Chromosomenkonstitution von Eltern und Nachkommen, und z. T. auch die Vitalität der verschiedenen Genotypen vollständig analysierte Nachkommenschaften. Zwei von diesen Nachkommenschaften („IV" und „VII") bildeten außerdem den Anfang von „balancierten" nukleolenheterozygoten Linien, deren Herstellung das Hauptziel der Kreuzungen gewesen war (s. S. 277).

Spaltungszahlen und Chromosomenkonstitution der ausgewerteten „$F_{11}R$"-Nachkommenschaften sind in den Tabellen 1—3 wiedergegeben. Tabelle 1 gibt zunächst einen Überblick über die in der Embryonalentwicklung beobachtete Aufspaltung in lebensfähige und letale Embryonen. Die letalen gliedern sich in die nukleolenlosen (N^-) Zygoten, die an ihrer frühzeitig einsetzenden charakteristisch abweichenden Entwicklungsweise zu erkennen sind (der Nachweis, daß diese Embryonen tatsächlich keine Nukleolenbildungsorte besitzen, wird unten geführt), und solche, die in irgendeiner anderen Weise so in ihrer Entwicklung gestört sind, daß keine Larven aus der Eihülle schlüpfen, also alle sonstigen Embryonalletalen. Für die Fragestellung dieser Arbeit interessiert bei den Spaltungsdaten die relative Häufigkeit der N^--Embryonen. Bei dem besprochenen Versuch waren Nachkommenschaften mit 25%, 12,5%

Tabelle 1. *Spaltungsergebnisse der „$F_{11}R$"-Nachkommenschaften im Embryonalstadium, geordnet nach der Nukleolenkonstitution der Eltern*

Gelege	Chromosomen der Eltern		Entwickelte Zygoten	N⁻	Sonstige Letale	Fragliche	% N⁻ + Fragliche
	2R	3L					
a) Kreuzungstypus 2R: $NN^- \times NN^-$ (2R: t/pN × t/pN) (Erwartung für N⁻-Zygoten: 25%)							
„IV"	t/pN × t/pN	pp × pp^k	686	132	137	—	19,3
„VI"	t/pN × t/pN	pp × pp^k	996	147[1]	1	13	16,1
„VII"	t/pN × t/pN	pp × pp^k	944	226	4	6	24,5
„VIII"	t/pN × t/pN	pp^k × pp^k	856	131	126	24	18,1
„XII"	t/pN × t/pN	p^kp^k × p/p^k (3R: tp × pp!)	833	223	133	—	26,7
„XVI"	t/pN × t/pN	p × pp^k	587	73	31	27	17,2
b) Kreuzungstypus 2R: NN^- × NN^-; 3L: N^-N^- × NN^- oder umgekehrt (2R t/pN; 3L pp × 2R t/pN; 3L tN/p) (Erwartung für N⁻-Zygoten: 12,5%)							
„II"	t/pN × t/pN	p^kp^k × tN/p	844	89	2	—	10,5
„XV"	t/pN × t/pN	p ? p ? × tN/p	753	68	143	16	11,2
„XI"	t/pN × t/pN	tN/p^k × pp^k	541	68	142	—	12,6
„XVI"	t/pN × t/pN	tN/p ? × pp^k	838	110	75	5	13,7
c) Kreuzungstypus 2R: NN^- × NN^-; 3L: NN^- × NN^- (2R t/pN; 3L tN/p × 2R t/pN; 3L tN/p) (Erwartung für N⁻-Zygoten: 6,25%)							
„V"	t/pN × t/pN	tN/p ? × tN/p ?	841	37	194	12	5,8
„XIII"	t/pN × t/pN	t/Np ? × tN/p ?	901	44	6	8	5,7

[1] Zählfehler möglich.

und 6,25% N^--Embryonen zu erwarten, entsprechend den 3 Kreuzungstypen, die am Kopf der Tabellen 1a, 1b und 1c symbolisiert sind. Tatsächlich wurden diese theoretischen Häufigkeiten in vielen Fällen nicht erreicht, insbesondere nicht beim monohybriden Spaltungstypus (Tabelle 1a), selbst wenn man zu den sicher erkannten N^--Embryonen noch die fraglichen (durch Platzen oder Bakterienbefall unkenntlich gewordenen) Embryonen hinzufügt, wie es bei der Errechnung der Prozentzahlen geschehen ist. Die Abweichungen vom theoretischen Spaltungsverhältnis sind nicht zufälliger Natur: Im Fall des Geleges „IV" (Tabelle 1a, erste Reihe) z. B. $\chi^2 = 12$, $P < 0{,}01$. Auch Gelege „II" (Tabelle 1b) spaltet nicht eindeutig in dem hier erwarteten Verhältnis 7:1 ($\chi^2 = 2{,}8$, $P < 0{,}1$).

Die Verschiebung der Spaltungszahlen zu Ungunsten der N^--Zygoten bis auf weniger als 20% (bei einfacher Nukleolenheterozygotie) ist auch in den Nachkommenzuchten der in Tabelle 1a aufgeführten Gelege immer wieder beobachtet worden (Tabelle 2). Sie kann folgende Ursachen haben: 1. gerichtete Reduktion in der Oogenese, so daß mehr als 50% der ♀ Vorkerne das Chromosom (2R pN) mit dem Nukleolenbildungs-

Tabelle 2. *Embryonische Spaltungsdaten bei verschiedenen Kreuzungen von Nukleolenheterozygoten (Kreuzungstypus allgemein: 2 R t/pN × 2 R t/pN)*

Gelege[1]	Entwickelte Zygoten	N^-	Sonstige Letale	Fragliche	% N^- + Fragliche	χ^2	P
VII/1a	1213	270	213	23	24,2	0,4	0,5—0,6
VII/1b	1213	221	3	66	23,7	1,2	0,2—0,3
VII/1c	987	204	61	—	20,8	10,0	$<0{,}01$
VII/1d	872	169	165	—	19,5	14,6	$<0{,}01$
VII/1e	919	204	1	—	22,3	3,9	0,05
VII/1f	663	120	228	—	18,1	16,9	$<0{,}01$
IV/1k	582	133	94	5	23,7	1,6	0,2
VIII/1a	877	114	15	15	14,7	67	$\ll 0{,}01$
IV × VIII	877	149	2	13	18,4	29.5	$<0{,}01$
IV/5a	491	139	—	—	28,3	2,8	$<0{,}1$
IV/5b	305	60[2]	71	—	19,7	4,5	$<0{,}05$
IV/5c	352	90	4	—	25,7	0,05	0,8—0,9
IV/5d	432	87	68	—	20,1	5,5	0,02—0,01
IV/5e	592	132	182	—	22,3	2,3	0,2—0,1
IV/5f	618	175	17	—	28,3	3,5	0,1—0,05

[1] Die Gelege VII/1ff. und IV/5 ff. sind Schwestergelege und gehören der 1. bzw. der 5. Generation nach Herstellung der balancierten Linien „VII" und „IV" an.
[2] Zählfehler ± 5 möglich.

ort erhalten; 2. die N^--Spermien sind entweder z. T. funktionsunfähig oder generell in ihrer Funktion (Beweglichkeit, Eindringen in das Ei usw.) benachteiligt. Die Entscheidung zwischen diesen Alternativen ist schwierig, sie setzt voraus, daß es gelingt, in entsprechend angesetzten Testkreuzungen sämtliche Zygoten einer Stichprobe bis zum 4. Larvenstadium aufzuziehen, wo man dann ihre Chromosomenkonstitution in den Speicheldrüsenkernen feststellt. Derartige Untersuchungen sind noch im Gange. Die zuletzt angeschnittenen Fragen, die an sich nur allgemein-cytogenetisches Interesse besitzen, könnten auch für das Nukleolenproblem Bedeutung erlangen, wenn es sich zeigen ließe, daß die Abweichungen von der reinen Mendelspaltung in Spermato- oder Oogenese etwas mit dem Besitz von bzw. dem Mangel an Nukleolenbildungsorten zu tun haben.

Alle Überlegungen setzen selbstverständlich eines voraus: Daß nämlich allein die sog. „N^--Embryonen" diejenigen Zygoten repräsentieren, die keinen normalen Nukleolenbildungsort besitzen. Es muß also die Möglichkeit ausgeschlossen sein, daß ein Teil der N^--Zygoten doch lebensfähig ist oder andersartige Letalembryonen bildet (etwa weil eine bestimmte genetische Konstitution die Entstehung von „Ersatz"-Nukleolen erlauben würde). Aus diesem Grunde wurden aus einigen der „F_{11} R"-Nachkommenschaften abgezählte Stichproben von geschlüpften Eilarven entnommen und bis zum 4. Larvenstadium aufgezogen. Die Anzahl der Überlebenden sowie die Chromosomenkonstitution ihrer Speicheldrüsenkerne sind in der Tabelle 3 zusammengestellt.

Tabelle 3. *Chromosomenkonstitution überlebender Larven von Stichproben aus „$F_{11}R$"-Nachkommenschaften* (Chromosomenkonstitution der Eltern s. Tabelle 1a—c)

Gelege	Angesetzte Eilarven	Überlebende IV. Stadium	Geprüft	Anzahl der geprüften Larven mit										
				1L			2R		3L			4		
				pp	pp^1	p^1p^1	t/pN	pN pN	pp	pp^k	p^kp^k	tt	tp	pp
a) Kreuzungstypus „2R t/pN × 2R t/pN"														
„IV"	190	127	40[1]	16	23	1	40	0	16	24	—	13	17	10
„VI"	150	125	107	34	49	24	64	43	44	63	—	19	56	28[3]
„VII" a)	200	127	110	30	60	14[2]	110	0	54	56	—	29	46	31[3]
b)	360	236	—											
„XII"	150	132	70	18	46	6	41	29	—	68	2[4]	13	45	12
„XVI"	105	85	20	7	12	1	10	10	7	13	—	4	15	1

[1] Nur die größten Larven. [2] + 6 fragliche. [3] + 4 fragliche homozygote. [4] Spaltung in 3R: tp:pp = 45:25 (Eltern 3R tp × pp).

b) Kreuzungstypus „2R t/pN × 2R t/pN; 3L tN/p" oder umgekehrt

Gelege	Angesetzte Eilarven	Überlebende IV. Stadium	Geprüft	Anzahl der geprüften Larven mit:												
				1L			2R			3L					4		
				pp	pp^1	p^1p^1	t/pN	t/pN	pN pN	pp	pp^k	p^kp^k	tN/p	tN/p^k	tt	tp	pp
„II"	350	206	185	48	96	41	33	117	35	—	64	—	—	121[2]	43	80	42
„III"[1]	140	114	96	31	63	2	9	87	0	26[3]	—	—	70[3]	—	20	51	25
„XI"	157	103	103	16	62	15	14	89	0	—	28	(4)[4]	38	33	20	58	25

[1] Fehlt in Tabelle 1b, da Anzahl der N⁻Zygoten unklar; aus 970 entwickelten Eiern schlüpften 444 Eilarven, also 54% Embryonalletalität. [2] Spaltung in 3R tp:pp = 121:64 (Eltern 3R pp × tp). [3] Nicht registriert, ob p oder p^k, jedoch p wahrscheinlicher; Spaltung in 3R tp:pp = 67:28 (+ 1 crossover) (Eltern 3R pp × tp). [4] 4 crossover-Tiere, d. h. 3R tp; 3 davon mit N; Spaltung in 3R tp:pp = 71:32 (Eltern 3R tp × pp).

c) Die Nukleolenkonstitution der Tiere aus Tabelle 3b

		2R		
		tt	t/pN	pN/pN
3L	tN/p	56	183	20
	pp	0	103	15

Das wichtigste Ergebnis dieser Untersuchung ist, daß Larven (des 3. und 4. Stadiums) ohne Nukleolenbildungsort nicht gefunden wurden. Etwaige verkappte Nukleolenlose müßten also spätestens als Larven des 1. oder 2. Stadiums gestorben sein; das ist aber aus anderen Gründen unwahrscheinlich (vgl. S. 281 ff.).

Gewiß läßt die Differenz zwischen angesetzten Eilarven und überlebenden bzw. untersuchten Larven im 4. Stadium in jedem einzelnen Falle genügend Raum zu Unterbringung einer hypothetischen Letalklasse, doch wird das Absterben eines Teiles der Larven (stets mindestens 10%) ohne Zwang anders erklärt: Zunächst sind bei der Aufzucht von *Chironomus*-Larven Unglücksfälle nie ganz auszuschließen (Hängenbleiben der Larven an der Wasseroberfläche, Bakterienbefall, gegenseitiges Anfressen usw.); zweitens weisen die Daten der Tabelle 3 auf die Existenz einer ganzen Reihe von Letal- und Subtivalfaktoren (bzw. -chromosomen) hin, die mit der Nukleolenbildung nichts zu tun haben, und denen man den größten Teil der Larven- und zudem die gesamte Embryonalsterblichkeit zuschreiben kann. In den Zuchten „IV“, „VII“, „III“ und „XI“ ist beispielsweise der Chromosomenarm 2R pN, d.h. gerade derjenige, der im Gegensatz zu seinen homologen Partner 2Rt einen Nukleolenbildungsort enthält, rezessiv letal: Die Homozygoten 2R pN/pN sterben als Larven kurz vor oder nach dem Schlüpfen (was aus einem Vergleich der Daten von Tabelle 1 und 2 mit jenen der Tabelle 3 folgt). Mit den Zuchten „IV“ und „VII“ ist also ohne weiteren Aufwand das Ziel verwirklicht, „balancierte“ Stämme von Nukleolen-Heterozygoten herzustellen: Da sowohl die Homozygoten 2Rt/t (N^--Zygoten) als auch die Homozygoten 2R pN/pN absterben, bleiben zur Weiterführung des Stammes stets allein die Heterozygoten übrig, und in jeder Nachkommenschaft spalten (theoretisch) $^1/_4$ N^--Zygoten heraus. Eine Durchbrechung der balancierten Heterozygotie durch crossing-over braucht nicht befürchtet zu werden, denn der Chromosomenarm 2Rt unterscheidet sich (bei den Stämmen „IV“ und „VII“) von seinem Partner 2R pN durch zwei einander übergreifende Inversionen, die den Austausch zwischen Nukleolenbildungsort und hypothetischen Letalfaktoren wirkungsvoll verhindern. Noch nach jetzt etwa 10 Generationen unkontrollierter Weiterzucht ist der Stamm „IV“ balanciert.

Die anderen aus den Daten der Tabelle 4 ablesbaren Letal- und Subvitalwirkungen bestimmter Genotypen können im Rahmen dieser Arbeit nicht ausführlich erörtert werden. Es sei nur auf zwei deutliche Effekte aufmerksam gemacht: Der Chromosomenarm 1L von *pallidivittatus* kann die in der Tabelle kurz als p^1 bezeichnete Inversion In(p)1L-1 (Beermann 1955) enthalten. Die Homozygoten p^1p^1 sind als Larven (und als Imagines) gegenüber pp^1 und auch pp stark benachteiligt, was sich in verminderter Wachstumsgeschwindigkeit und geringerer Größe äußert. Präpariert man also routinemäßig zunächst die Speicheldrüsenchromosomen der größten Larven einer Kultur, so erscheint die Klasse der Homozygoten p^1p^1

gar nicht oder nur in einzelnen Tieren (vgl. Tabelle 3a, „IV", „XII" und „XVI"). Je höher die Überlebensrate der Stichproben, und je vollständiger ihre Prüfung, desto größer ist die Anzahl der (zum Schluß doch noch heranwachsenden) homozygoten p^1p^1-Individuen. — Ein zweiter, noch interessanterer Fall von Letalität findet sich im Chromosom 3. Der linke Arm dieses Chromosoms kann die komplexe, dreifache Inversion In(p)3L-k1 (kurz als p^k bezeichnet) aufweisen. Homozygote p^kp^k sind letal, sofern gleichzeitig im rechten Arm des Chromosoms 3 Homozygotie für die *pallidivittatus*-Standardanordnung 3Rp vorliegt. Ist dagegen (infolge crossing-over) in 3R die heterozygote Kombination 3R p/t verwirklicht, so können die Homozygoten 3L p^k/p^k durchkommen. Es handelt sich also um einen komplementären Letaleffekt.

3. Entwicklung und Cytologie der N⁻-Embryonen

a) Entwicklung. Wie vorstehend gezeigt worden ist, erhält man nach der Kreuzung von Nukleolen-Heterozygoten eine Klasse von Letalembryonen, welche die Klasse der N⁻-Zygoten repräsentieren muß. Zunächst sei die Entwicklung dieser Embryonen skizziert (nach unveröffentlichten Daten von H. Krohn, Marburg). Die Beschreibung stützt sich auf Schnitte und auf die direkte Beobachtung lebender Embryonen. Das Chorion von *Chironomus*-Eiern ist völlig durchsichtig; außerdem entwickeln sich die Eier eines Geleges alle gleichzeitig, so daß Abweichungen im Entwicklungsverhalten einzelner Embryonen leicht erkannt werden können.

Bis zum Blastodermstadium ist in der Entwicklung von normalen und von N⁻Zygoten kein Unterschied zu bemerken; alle Eier haben die Ausdifferenzierung des Blastoderms (bei 18° C) etwa 15 Std nach der Ablage abgeschlossen (Abb. 7, links oben). Auch der Keimstreif der N⁻-Zygoten (Abb. 7, Mitte oben) wird zunächst normal angelegt (18 Std nach Ablage). Erst dann (22—24 Std) kommt es zu deutlichen morphologischen Abweichungen (Abb. 7, rechts oben). Der N⁻-Embryo beginnt sich in der Längsachse zu kontrahieren, die Seitenplatten des Kopfes schließen sich nicht, der Vorderkopf wächst nicht und verkümmert (26 Std), die anfangs angelegte Segmentierung verliert sich wieder, der Keimstreif dehnt sich und wird dabei in unregelmäßiger Weise in den Dotter hineingestaucht (31—37 Std), um sich schließlich (45 Std) unter weiterer Verkürzung des Embryos in eine halbe Schraubenwindung zu legen. Pharynx, Mitteldarm, oder auch nur Andeutungen von Entoderm fehlen ebenso wie das Nervensystem. Die Entwicklungsstörung beginnt also, allgemein gesagt, als Störung der Gastrulation. Das weitere Schicksal der Letalembryonen ist gekennzeichnet durch eine fortschreitende Desorganisation, bzw. ein Stehenbleiben auf der genannten Entwicklungsstufe (Abb. 8), keine Organbildung, manchmal Andeutungen von Muskulatur, Ausbildung rudimentärer Kopfanhänge, vielleicht auch rudimentärer Ganglien. Der Dotter wird anscheinend nicht verbraucht. Trotz aller

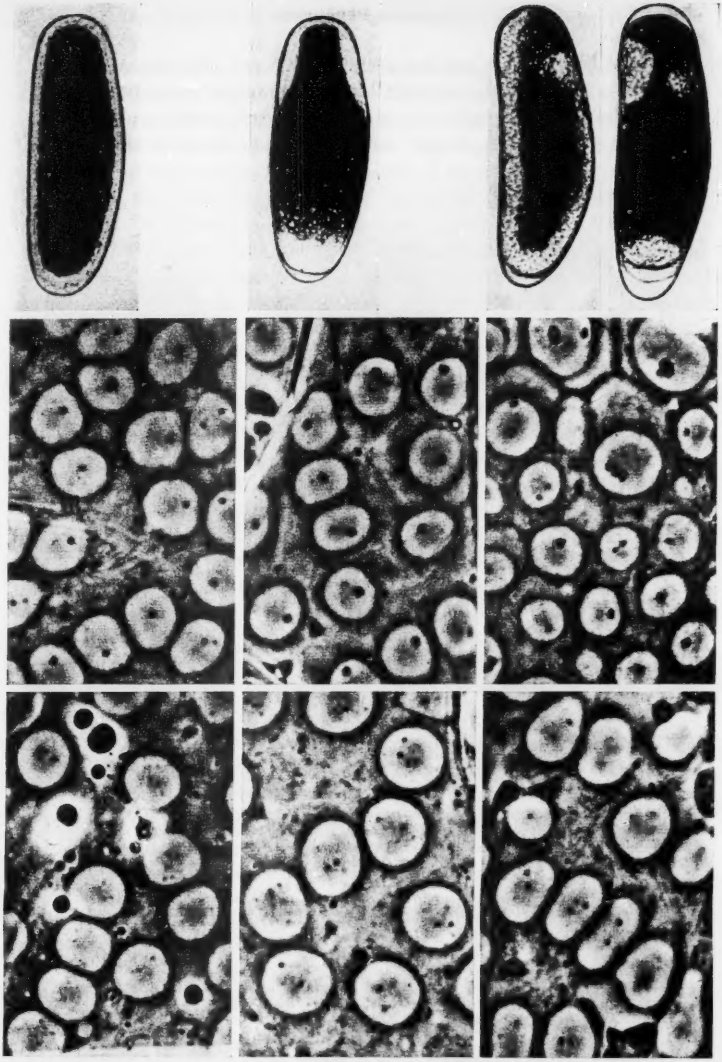


Abb. 7. Kerne von Nukleolenlosen (unten) und von normalen Geschwisterembryonen (Mitte). Oben: Das untersuchte Embryonalstadium. Links Blastoderm fast vollendet (Alter 12 Std bei 18° C); Mitte: Keimstreif (etwa 16 Std bei 18° C); rechts beginnende Segmentierung (etwa 23 Std bei 18° C), mit den ersten deutlichen Abweichungen der Nukleolenlosen (Embryo ganz rechts). Embryonen zur Nukleolendarstellung lebend in Wasser zerdrückt, Phasenkontrast. Embryonen 150 ×, sonst 1330 ×

Störungen können sich auf den Seitenplatten des „Kopfes" der Letalembryonen 2 oder 4 pigmentierte Flecken herausdifferenzieren (Abb. 8, rechts), die als die larvalen Augen gedeutet werden müssen: Sind auch Lage und Form dieser „Augen" sehr variabel, so ist doch das Pigment

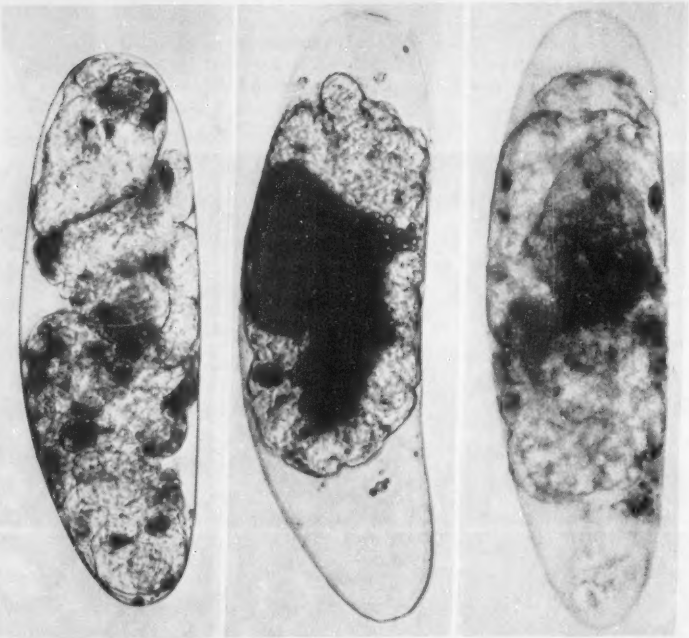


Abb. 8. Links: Schlüpfreife normale Larve, noch im Chorion. Mitte: Nukleolenloser Schwesterembryo gleichen Alters (etwa 70 Std bei 18° C). Rechts: Älterer nukleolenloser Embryo mit deutlichen Ocellen (etwa 80 Std alt, Schwestertiere längst geschlüpft). Lebend, 300 ×

das gleiche wie in normalen Larvenaugen. Charakteristisch für die Letalembryonen ist schließlich — zu einem Zeitpunkt, wenn die Normalembryonen längst als Larven geschlüpft sind — die beträchtliche Streckung und Dehnung des Chorions, die bei unbefruchteten Eiern und bei anderen Letalembryonen noch nicht beobachtet worden ist.

Die Mitoseaktivität der N⁻-Letalembryonen entspricht in den äußerlich normal ablaufenden Phasen ihrer Entwicklung, also bis zum späten Blastoderm, selbstverständlich derjenigen der normalen Embryonen. Über die spätere Entwicklung läßt sich soviel sagen, daß auch in deutlich desorganisierten Embryonen noch Mitosen ablaufen. Die Entwicklungsstörung basiert also nicht auf einer Mitosestörung.

b) Cytologie. Die cytologische Untersuchung sollte in erster Linie die Frage beantworten, ob die als „N⁻-Embryonen", also als Embryonen ohne Nukleolenbildungsorte gedeuteten Letalembryonen wirklich Zellkerne ohne normale Nukleolen besitzen. Eine Schwierigkeit liegt in der geringeren Größe der zu untersuchenden Zellkerne: Embryonale Zellkerne von *Chironomus* haben im fixierten Schnittpräparat einen Durchmesser von höchstens 5 μ, ihre Nukleolen einen Durchmesser von etwa 0,5—1 μ. Es gelang nicht, in diesen Dimensionen die Nukleolen durch Färbung verläßlich darzustellen. Da die Paraffinschneidetechnik die rasche Klassifizierung zahlreicher Embryonen ohnehin nicht erlaubt, so wurde sie ganz zugunsten einer einfachen Quetschtechnik aufgegeben: Die Eier bzw. Embryonen werden in einem Tropfen Leitungswasser unter dem Deckglas durch leichtes Andrücken zum Platzen gebracht. Weiteres leichtes Andrücken, mit einem Streifen Filtrierpapier als Zwischenlage, läßt das Zellmaterial sich in einer Schicht ausbreiten; gleichzeitig wird das überschüssige Wasser abgesaugt. Die Präparate werden sofort im Phasenkontrast (Ölimmersion) durchmustert. Sie halten sich einige Minuten. In dem stark hypotonischen Leitungswasser schwellen die Zellkerne augenblicklich an und erscheinen, bis auf die Nukleolen, optisch leer (Chromocentren, die ebenfalls sichtbar bleiben würden, kommen in den Zellkernen von *C. tentans* und *C. pallidivittatus* nicht vor). Auch kleinere Nukleolen sind auf diese Weise ihrer Zahl und Form nach sicher zu erfassen.

Mit dem angegebenen Verfahren konnten eine große Anzahl von normalen und letalen Embryonen in allen wichtigen Entwicklungsphasen auf ihre Nukleolenkonstitution hin geprüft werden. Dabei ergab sich zunächst, daß (erwartungsgemäß) die Zellkerne der frühen Furchungsstadien (vor der Herstellung des Blastoderms) keine Nukleolen ausbilden, auch nicht bei völlig normaler Chromosomenkonstitution. Nukleolen beginnen sich erst zu bilden, wenn die Kerne in das Keimhautblastem eingewandert sind, also während der Ausdifferenzierung des Blastoderms (dies und das folgende gilt für normale Embryonen). Die Nukleolen sind zunächst noch recht klein (Abb. 7); erst bei 24stündigen Embryonen erreichen sie ihre (für embryonale Zellkerne) charakteristische Größe und Form. Während die Nukleolen in den Anfangsphasen ihrer Entstehung noch kugelig erscheinen (Abb. 7, links und Mitte), sind sie später unregelmäßig geformt, lappig oder zerfranst (Abb. 7, rechts). Daß sie tatsächlich an den Nukleolenbildungsorten gebildet werden, folgt aus der von Anfang an zu beobachtenden Korrelation zwischen der Anzahl der Nukleolen einerseits und der Anzahl der Nukleolenbildungsorte andererseits. Bei *Chironomus tentans* z. B. zeigen die frühembryonalen Kerne erwartungsgemäß im Höchstfall 4 Nukleolen (bei 2 Paar Nukleolenbildungsorten), und in der Regel 2 Nukleolen. Bei *C. pallidivittatus* (mit 1 Paar Nukleolenbildungsorten)

finden sich höchstens 2, und in der Regel 1 Nukleolus. Bei den Nukleolenheterozygoten schließlich, die nur einen einzigen, heterozygoten Nukleolenbildungsort pro Zellkern besitzen, haben sämtliches Kerne nur einen Nukleolus.

Wie ist nun die Situation in den Kernen der „N^--Embryonen"? Betrachtet man zunächst N^--Embryonen in einem Alter, wo sie sich äußerlich bereits deutlich von ihren normalen Geschwistern unterscheiden (Abb. 7, unten rechts), so ist kein Zweifel möglich: Die Kerne enthalten keine normalen Nukleolen. An ihrer Stelle finden sich kleine, lichtbrechende, kugelige Körperchen in wechselnder Anzahl (3—5, oder auch mehr). Die mittlere Größe dieser Körnchen nimmt vom frühen Blastoderm, wo sie analog zu den normalen Nukleolen zum ersten Mal auftreten, allmählich zu (vgl. Abb. 7, untere Reihe). Ein Indiz für eine direkte Beteiligung bestimmter Chromosomenorte an dem Aufbau dieser „Pseudonukleolen" gibt es nicht.

Zuletzt ist die Frage zu beantworten, ob (im Hinblick auf die abweichenden Spaltungsdaten, vgl. Abschnitt 2) nach einer Kreuzung von Nukleolen-Heterozygoten ausschließlich die N^--Embryonen, oder auch einzelne andere Embryonen keine normalen Nukleolen in ihren Zellkernen aufweisen. Hierzu wurde eine Stichprobe von 24stündigen Embryonen, die alle einem Gelege aus dem Stamme IV angehörten, zuerst morphologisch und dann cytologisch klassifiziert. Von 210 Embryonen waren 147 äußerlich normal, und sie alle besaßen auch normale Nukleolen. 58 Embryonen wurden äußerlich als Nukleolenlose klassifiziert. Von ihnen besaßen 52 keine Nukleolen; bei den restlichen 6 liegt also eine Verwechslung mit einem anderen Letalfaktor vor. 5 Embryonen konnten äußerlich nur als abweichend, nicht als sicher nukleolenlos klassifiziert werden; von ihnen waren zwei echte N^--Embryonen. Auch in allen sonstigen Proben hat es sich bestätigt, daß allein die N^--Embryonen die Zygoten ohne Nukleolenbildungsort repräsentieren.

4. Die Bedeutung des Sondernukleolus am Chromosom 4

Es wurde schon erwähnt (S. 265), daß bestimmte 4. Chromosomen von *C. tentans* und *C. pallidivittatus* einen Nukleolenbildungsort besitzen, der einen Sondernukleolus von nahezu der Größe der normalen Nukleolen zu bilden vermag. Die Struktur des Sondernukleolus und sein sporadisches Auftreten machen es wahrscheinlich, daß er physiologisch den normalen Nukleolen nicht gleichwertig ist. Eine Möglichkeit, diese Frage zu prüfen, bieten die nukleolen-heterozygoten Stämme; durch Einkreuzen des mutanten 4. Chromosoms in diese Stämme muß es sich entscheiden lassen, ob die Funktionen der normalen Nukleolen in der Embryogenese ganz oder zu einem Teil durch den Sondernukleolus ersetzt werden können oder nicht.

Die Untersuchung zeigt, daß Zygoten, die wohl einen Sondernukleolus, aber keine normale Nukleolen besitzen (N^-; SN 4), ebenso absterben wie Zygoten ohne jeden Nukleolus (Tabelle 4). Die Entwicklung der N^--Letalembryonen mit oder ohne Sondernukleolus verläuft gleicnartig. Es ließe sich einwenden, daß die Sondernukleolen in der Embryogenese vielleicht noch nicht gebildet werden, und daß deshalb die Einkreuzung ihrer Bildungsorte ohne Wirkung bleibt. Der Einwand wird durch die cytologische

Tabelle 4. *Spaltungsdaten aus Kreuzungen mit dem Sondernukleolus am Chromosom 4*

Nukleolenkonstitution der Eltern	Gesamtzahl des untersuchten Embryonen	„N^{-}"[1]	Sonstige Letale	Fragliche	% N^-	E %[2]
(2R N^-/N; 4 SN/+)[2] .	849	215	10	10	25,2	6,25
2R N^-/N; 4 SN/+ × 2R N^-/N	212	59	—	—	27,7	12,5
2R N^-/N; 4 SN/+ × 2R N^-/N; 4 SN/+[3]	180	44	—	10	24,2	6,25 (12,5)[3]
2R N^-N; 4 SN/+ × 2R N^-/N; 4 ?	691	157	163	—	22,7	max. 12,5

[1] Vergl. Tabelle 1.

[2] Erwartungen für N^--Embryonen, für den Fall, daß der Sondernukleolus SN 4 die Funktion normaler Nukleolen ersetzen kann.

[3] ♂ nicht sicher 4 SN/+.

Untersuchung entkräftet (Abb. 9): Ebenso wie die normalen Nukleolen treten auch die Sondernukleolen zum erstenmal im Blastodermstadium auf und werden mit fortschreitender Embryonalentwicklung immer deutlicher. Die Sondernukleolen unterscheiden sich von den normalen Nukleolen in den Embryonen durch die gleichen Merkmale wie in den larvalen Speicheldrüsenkernen. Sie sind im Gegensatz zu den normalen Nukleolen kugelrund und stärker lichtbrechend; ihre Größe schwankt beträchtlich von Kern zu Kern, auch in kleinen Zellarealen.

5. Experimentelle Unterteilung von Nukleolenbildungsorten

Nach der Bestrahlung von *C. pallidivittatus*-♂♂ mit 5000 r Röntgenstrahlen fand sich unter den Larven der F_1 mit Chromosomenmutationen 1 Tier, bei welchem der Nukleolenbildungsort (in 2R) in 2 funktionsfähige Teilstücke zerlegt war. Ein Chromosomenbruch im Nukleolenbildungsort war mit einem anderen Chromosomenbruch in Form einer reziproken Translokation rekombiniert, und an beiden Rekombinationsorten hatte sich ein Nukleolus gebildet. Dieser Fall gab Anlaß zur Suche nach weiteren derartigen Fällen bei der F_1 von bestrahlten *palli-*

divittatus-♂♂. Um die Weiterzucht von etwaigen weiteren Mutationen der gewünschten Art zu sichern, wurden die F_1-Tiere erst nach der Produktion von Nachkommen cytologisch untersucht (Malphigigefäß-Chromosomen). Damit sollte es ermöglicht werden, die Leistungen der Teilstücke des Nukleolenbildungsortes durch Einkreuzen in nukleolenheterozygote Stämme einzeln zu prüfen.

Unter etwa 100 geprüften F_1-Imagines von bestrahlten *C. pallidivittatus*-♂♂ trat 1 Tier mit einer neuen „Nukleolusfragmentation" auf

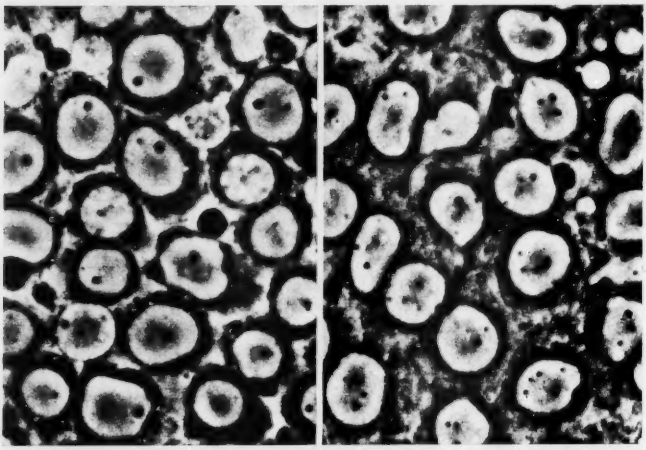


Abb. 9. Kerne von etwa 24 Std alten Letalembryonen ohne normalen Nukleolenbildungsort, jedoch links mit Sondernukleolus. Rechts Schwesterembryo ohne jeden Nukleolenbildungsort. Embryonen lebend in Wasser zerdrückt, Phasenkontrast. 1400 ×

(Mutation „8"). Die Mutation, die noch als Stamm gehalten wird, besteht in der Verlagerung („shift") eines kurzen Abschnitts im Chromosomenarm 2R an eine andere Stelle weiter distal im gleichen Chromosomenarm (Abb. 10); sie ist also das Ergebnis dreier Chromosomenbrüche. Von proximal nach distal liegt der erste Bruch im Abschnitt 11A, der zweite genau im Nukleolenbildungsort, und der dritte im Abschnitt 17A (vgl. die Chromosomenkarte bei BEERMANN 1955); das durch die Brüche 1 und 2 begrenzte Fragment ist an der dritten Bruchstelle wieder eingefügt, und die durch das Herausbrechen des Fragmentes zwischen 1 und 2 entstandene Lücke ist durch Zusammenschluß der offenen Bruchflächen wieder verheilt. Wesentlich ist nun die Tatsache, daß beide durch den Bruch 2 entstandenen Bruchenden auch in der neuen Anordnung einen Nukleolus bilden, wodurch eben bewiesen wird, daß Bruch 2 tatsächlich innerhalb des Nukleolenbildungsortes liegt. Von

den beiden Nukleolen ist der distal gebildete stets kleiner als der proximale. Das entspricht dem mikroskopisch erkennbaren Größenverhältnis der beiden Bruchstücke des Nukleolenbildungsortes (Abb. 10): Gegenüber dem normalen Bildungsort (N) ist das proximal gelegene

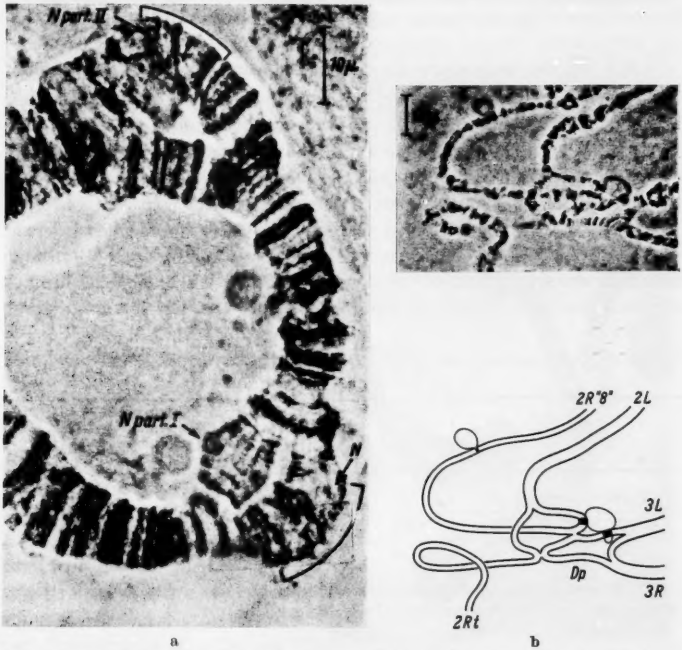


Abb. 10a u. b. Röntgeninduzierte Teilung eines Nukleolenbildungsortes (*2R* von *C. pallidivittatus*) in 2 ungleiche Partialbildungsorte: Mutation „8" (vgl. Text). a Chromosom *2R* in der Speicheldrüse einer Heterozygoten *pall.* normal/*pall.* „8". Verlagerung (shift) eines kurzen Abschnittes mit einem Stück des Nukleolenbildungsortes nach weiter distal (Markierungsstriche). b Malpighigefäßchromosomen einer Heterozygoten vom Typ 2 R *pall.* „8"/*tent.*; 3 L *pall.* „Dp"/*pall.* normal. „*Dp*" Transposition des Partialbildungsortes I (Mut. „8") in das Chromosom 3. *N* normaler Nukleolenbildungsort, *N part. I* = der größere Partialbildungsort, *N part. II* = der kleinere Partialbildungsort. Orcein-Essigsäure, bzw. Orcein Milchsäure, Phasenkontrast. Links 1200 ×, rechts 650 ×

Teilstück (N part. I) nur unmerklich verkürzt (höchstens um $^1/_5$). Das distale Teilstück (N part. II) ist mikroskopisch gerade eben als dünne blasse Querscheibe erkennbar.

Die Ergebnisse zeigen, daß eine ungleiche Teilung des Nukleolenbildungsortes funktionsfähige Teilstücke liefern kann. Um zu entscheiden, ob beide „Partialbildungsorte" für sich allein immer noch qualitativ

dasselbe leisten wie ein ungeteilter Nukleolenbildungsort, mußte zunächst die Koppelung der 2 Partialbildungsorte — beide liegen ja im gleichen Chromosomenarm 2R — durch crossing-over oder durch erneute Translokation aufgehoben werden. Nur der zweite Weg erwies sich als gangbar. Die Mutation „8" wurde zunächst in einen nukleolenheterozygoten Stamm eingekreuzt, so daß ein neuer nukleolen-heterozygoter, balancierter Stamm entstand mit der Konstitution 2R p„8"N/tN$^-$ × („8/N$^-$"); die balancierte Heterozygotie wird hier dadurch gewährleistet, daß die Homozygoten „8/8" letal sind. ♂♂ dieses Stammes wurden mit 5000 r bestrahlt.

Eines der F_1-Tiere wies eine Translokation vom gewünschten Typus auf: Der proximale Partialbildungsort (N part. I) (zusammen mit einem ihn einschließenden kurzen Chromosomenabschnitt) war vom Chromosomenarm 2R in die Mitte des Chromosoms 3 transponiert (wiederum ein Dreibruchereignis). Der neue Mutationsstamm („Tp/N$^-$") besitzt also 2 heterozygote Partialbildungsorte, die in verschiedenen Chromosomen liegen und deshalb im Kreuzungsexperiment frei rekombinierbar

Tabelle 5. *Spaltungsdaten bei Kreuzungen von „Tp/N$^-$" mit „8/N$^-$" oder „IV"* (vgl. Text)

Kreuzung	Untersuchte Embryonen	„N$^-$"	Nicht schlüpfende Larven	Sonstige Letale[1]	Fragliche	% N$^-$
♀ „Tp/N$^-$" × ♂ „8/N$^-$" .	1236	185	346	32	—	15,0
♀ „8/N$^-$" × ♂ „Tp/N$^-$" .	1099	129	266	54	—	11,7
♀ „IV" × ♂ „Tp/N" . .	1174	142	267	95	—	12,1

[1] Umfassen mindestens 3 verschiedene Klassen, die sich wohl alle durch crossing over im Bereich der transponierten Abschnitte erklären.

sind. Einige Daten von Kreuzungen dieses Stammes mit den einfach heterozygoten Nukleolenstämmen „IV" und „8/N$^-$" sind in Tabelle 5 zusammengestellt. In den Kreuzungen sollten theoretisch $^1/_8$ (12,5%) N$^-$-Zygoten herausspalten. Die Übereinstimmung der gefundenen Zahlen von N$^-$-Embryonen mit der Erwartung ist überraschend gut, wenn man bedenkt, daß die Tiere des Stammes „Tp/N"$^-$ neben Tp- und N$^-$-Gameten auch solche mit einer Defizienz (Df) in 2R oder mit einer Duplikation (Dp) im Chromosom 3 ausbilden (alle 4 Gametensorten im Verhältnis 1:1:1:1). Das läßt eine hohe embryonale Letalität bei den Nachkommen erwarten, wobei zunächst nicht auszuschließen ist, daß eine der so entstehenden Letalklassen morphologisch den N$^-$-Embryonen gleicht. Man erkennt aber aus den Zahlen in Tabelle 5, daß neben der Klasse der N$^-$-Embryonen nur eine weitere in sich einheitliche größere

Letalklasse auftritt, nämlich die der nicht oder zu spät schlüpfenden Eilarven. Diese Klasse macht etwa 25% aus und repräsentiert so gut wie sicher die Df-Heterozygoten (2R „8"/Df oder N^-/Df), denn Dp-Zygoten (2R „8"/N^-; 3Dp/N^- oder N^-/N^-; Dp/N^-) sind als Larven lebensfähig, wie die Untersuchung der Speicheldrüsenchromosomen der überlebenden Larven gezeigt hat (s. auch weiter unten).

Mit den vorstehenden Kreuzungsresultaten ist über die Leistungsfähigkeit der beiden partiellen Nukleolenbildungsorte in den Mutationsstämmen „8" und „Tp" bereits alles Wesentliche gesagt. Die Dp-Heterozygoten zeigen ernsthafte Entwicklungsstörungen überhaupt nicht, wenn sie neben dem Partialbildungsort „N part. I" sonst keinen Nukleolenbildungsort besitzen (Abb. 11). Als Larven und Imagines sind Zygoten dieser Konstitution (N^-/N^-; DpN part. I/N^-) allerdings in ihrer Vitalität etwas benachteiligt, aber das liegt eher an der Homozygotie für 2Rt; die Imagines des gleichen Genotypus sind fertil. Andere Kreuzungen haben gezeigt, daß sogar homozygote Dp-Larven überleben (2Rtt; 3Dp/Dp). Was nun die vermutlichen Df-Zygoten (die Klasse der nicht schlüpfenden Larven) betrifft, so zeigen jedenfalls auch sie keine frühembryonalen Entwicklungsstörungen. Die spätembryonale Letalität kann nur eine Folge der Defizienz an sich, aber nicht eine Folge davon sein, daß die Df-Zygoten z. T. nur den einen Partialbildungsort „N part. II" und sonst keinen Nukleolenbildungsort besitzen; denn dann wären nicht 25%, sondern nur 12,5% nicht schlüpfende Eilarven zu erwarten. Es kann demnach als erwiesen gelten, daß jedes der beiden hier untersuchten röntgeninduzierten Fragmente des Nukleolenbildungsortes 2RpN physiologisch vollwertige Nukleolen produziert.

Abb. 11. Speicheldrüsenchromosomen einer Larve, die außer dem heterozygoten Partialbildungsort in 3L („Dp") keinen weiteren Nukleolenbildungsort besitzt. Orcein-Essigsäure, 480 ×

Diskussion

Die vorliegende Arbeit ging von der bislang noch offenen Frage aus (vgl. das Referat von VINCENT 1955), ob und wie weit Nukleolen und ihre Bildungsorte überhaupt lebenswichtige Zell-Organe sind. Die Untersuchungen geben darauf eine klare Antwort: *Chironomus*-Zygoten ohne Nukleolenbildungsort im Genom entwickeln sich zu Letal-Embryonen. Dies stimmt mit den Befunden von ELSDALE, FISCHBERG und SMITH (1958) am Krallenfrosch *Xenopus* vollständig überein und darf daher wohl verallgemeinert werden. Bei *Chironomus* setzt die Entwicklung zu lebens- und fortpflanzungsfähigen Tieren die Anwesenheit von mindestens einem „echten" Nucleolenbildungsort voraus, der auch heterozygot und auch als Partial-Bildungsort vorliegen kann. Verschiedene Nukleolenbildungsorte (mit Ausnahme des akzessorischen Bildungsortes, vgl. S. 282) können sich gegenseitig vertreten. Daraus folgt, daß die Letalität der völlig „nukleolenlosen" Zygoten allein auf ihrer Unfähigkeit beruht, echte Nukleolen zu bilden; sie kann nicht als Folge von genetischen Defizienzen im Bereich bzw. der Nachbarschaft der Nukleolenbildungsorte gedeutet werden. Dieser Nachweis ist bei *Xenopus* nicht mit der gleichen Schärfe zu führen. Die echten Nukleolen, die ausschließlich durch die Aktivität der echten Nukleolenbildungsorte zustandekommen, sind also lebenswichtig. Im folgenden soll besprochen werden, worin die Lebenswichtigkeit der Nukleolen im einzelnen begründet liegen könnte.

Einige Kriterien für die Bedeutung der Nukleolen im Zellstoffwechsel liefern die Entwicklungsleistungen der nukleolenlosen Embryonen. Die Entwicklung verläuft bis zur Anlage des Keimstreifs (Gastrulation) ohne erkennbare Besonderheiten. Erst in diesem Stadium also beginnen die Nukleolen unentbehrlich zu werden. Dabei bleiben die Einzelzellen aber noch lange am Leben und teilen sich auch noch. Demnach scheinen wesentliche zellphysiologische Elementarvorgänge, z. B. die energieliefernden Reaktionen, ohne Nukleolen normal zu funktionieren. Das gilt auch für spezifische Syntheseleistungen, z. B. die Bildung von Augenpigment. Diese Schlußfolgerungen werden bekräftigt durch die z. T. noch weiter gehenden Entwicklungsleistungen der nukleolenlosen Embryonen von *Xenopus*, wie sie ELSDALE, FISCHBERG und SMITH beschrieben haben. Das Schädigungs-„Syndrom" der nukleolenlosen *Xenopus*-Embryonen bzw. -Larven ist bezeichnenderweise das gleiche wie es nach UV-Bestrahlung oder einfach bei Überbevölkerung der Kulturschalen auftritt.

Auch ein so komplizierter Vorgang wie die Mitose läuft ohne Nukleolen noch viele Zellgenerationen hindurch normal ab. Dies bestätigen wiederum die Ergebnisse von ELSDALE et al. (1958) an *Xenopus*, sowie Beobachtungen von v. BORSTEL und REKEMEYER (1959) an Nullo-XY-Zygoten von *Drosophila*. Die Beobachtung von GAULDEN und PERRY (1959), daß Neuro-

blasten, deren Nukleolus in der mitotischen Phase durch UV-Strahlenstich aufgelöst wurde, eine permanente Mitosehemmung erleiden, muß also anders gedeutet werden. Im übrigen erledigt sich dieser Punkt durch die ziemlich allgemein (mit Ausnahme der Säuger) geltende Regel, daß die Kerne tierischer Eier in den ersten Furchungsstadien vor der Bildung der Blastula schon normalerweise keine Nukleolen ausbilden (Literatur bei VINCENT 1955), und daß trotzdem gerade in dieser Entwicklungsphase die kürzesten Mitosezeiten erreicht werden (z. B. bei *Drosophila* 10 min pro Mitosezyklus).

Bei der Beurteilung der Leistungen von nukleolenlosen Zellen im Embryo hat man mit den besonderen Verhältnissen im tierischen Ei zu rechnen. Genetische Defekte, die sonst vielleicht unmittelbar letal wirken, könnten durch von der Mutter stammende Reserven im Embryo auf einige Zeit überbrückt und damit phänotypisch verdeckt werden. Aber auch die Möglichkeit der Überbrückung läßt eine Aussage über Art und Ausmaß des Schadens zu, wenn man verschiedene Letalfaktoren miteinander vergleicht. Dies gilt insbesondere für den Nukleolen-Defekt, denn er manifestiert sich mit Sicherheit in allen Zellen, ohne Rücksicht auf Zell-Charakter und Zell-Funktion, und wahrscheinlich auch in allen Zellen mit den gleichen Folgen. Trotzdem wirkt das Fehlen der Nukleolen weniger drastisch als einige bei *Chironomus* spontan aufgetretene oder durch Bestrahlung erzeugte andere Letalfaktoren, darunter auch einfache Letalmutationen ohne sichtbare Änderungen der Chromosomenstruktur. Alles deutet deshalb darauf hin, daß die Funktion des Nukleolus eine indirekte und allgemeine, möglicherweise regulierende ist, die aber dennoch auf lange Sicht von keiner Zelle entbehrt werden kann.

Ein fundamentaler Vorgang, dessen Unterbindung oder Ausbleiben nicht unmittelbar letale Folgen hat, ist das Wachstum. Der Gedanke eines Zusammenhanges von Nukleolen und Wachstum ist nicht neu und kann sich auf zahlreiche Beobachtungen an wachsenden Zellen stützen (vgl. z. B. CASPERSSON 1950). Vielleicht verursacht das Fehlen normaler Nukleolen eine allgemeine Störung des Zellwachstums. „Störung des Wachstums" kann bedeuten: 1. Störung oder gänzliches Fehlen der Synthese eines einzelnen Proteins, das in allen Zellen des Individuums produziert und gebraucht wird; 2. Störung oder Ausfall der gesamten Proteinsynthese; 3. Störung der Koordination verschiedener Proteinsynthesen. Die erste Möglichkeit schließt als Konsequenz die zweite oder die dritte mit ein.

Man kann zunächst annehmen, daß am Nukleolenbildungsort eine Ribonukleinsäure (RNS) entsteht, die als Informationsüberträger („template") für die Synthese eines einfachen Strukturproteins im Cytoplasma dient, z. B. für die Synthese des sog. „Trägerproteins" der Paladeschen Ribonukleoprotein-Partikel. In diesem Fall würde das Fehlen der Nukleolenbildungsorte bewirken, daß neues „Ergastoplasma" in den embryonalen

Zellen nicht mehr gebildet wird, sobald der mütterliche Vorrat an Trägerprotein verbraucht ist. Die Differenzierung (= Synthese zellspezifischer Proteine) wäre nicht gehemmt, wohl aber das Wachstum (= Vermehrung der Syntheseorte im Plasma). Die Entstehung des Nukleolus selbst könnte als zeitweilige Ausfällung des Trägerproteins durch die nukleoläre RNS verstanden werden. Ein solches Präzipitat, das an der Peripherie ständig abgebaut und vom Nukleolenbildungsort her ständig aufgefüllt würde, wäre gleichbedeutend mit einem Reservoir von Trägerprotein (und der zugehörigen RNS).

Die zweite Annahme (im Rahmen der drei oben genannten Möglichkeiten) geht dahin, daß die am Nukleolenbildungsort hergestellte RNS nicht als Träger von Information für die Synthese eines Proteins dient, sondern andere Funktionen im Proteinstoffwechsel erfüllt. Vielleicht ist die Nukleolen-RNS die niedermolekulare, „lösliche" RNS (oder deren hochmolekulare Vorstufe), die nach den neueren Erkenntnissen über die Protein-Biosynthese die einzelnen Aminosäuren an die richtigen Plätze der hochmolekularen RNS-„templates" (in den den Ribonukleoprotein-Partikeln des Ergastoplasmas) dirigiert. Fiele die Produktion dieser löslichen RNS aus, so müßte das in kurzer Zeit zum Zusammenbruch der gesamten Proteinsynthese, also auch der Synthesen der spezifischen Proteine, führen. Man würde dann annehmen, daß die nukleolenlosen Embryonen größere Vorräte von löslicher RNS von der Mutter her besitzen. Die zeitweilige Ausfällung der nukleolären RNS durch Protein wäre wiederum als Anlegung eines Reservoirs zu verstehen; Herkunft und Bedeutung des ausfällenden Proteins blieben offen. Es müßte sich in jedem Fall um ein einfaches, in allen Zellen des Körpers gebildetes Protein (z. B. wiederum das Trägerprotein, vgl. BRENNER 1959) handeln, dessen Speicherung vielleicht ebenso wesentlich oder wesentlicher wäre als die der nukleolären RNS selber (der Nukleolus besteht immerhin zu rund 95% seiner Trockenmasse aus Protein!).

Drittens mag die am Nukleolenbildungsort gebildete RNS überhaupt keine andere Aufgabe haben als das Nukleolenprotein in Form einer temporär unlöslichen Verbindung auszufällen und so zu speichern. Dann läge die Betonung allein auf der Speicherung des Proteins. Die Letalität der N^--Embryonen wäre in diesem Fall vielleicht als Folge einer Koordinationsstörung aufzufassen, hervorgerufen durch Schwankungen im Angebot des nukleolären Proteins.

Die vorstehend diskutierten Hypothesen schreiben dem Nukleolenbildungsort zwei Funktionen zu, nämlich 1. die Synthese einer bestimmten RNS und 2. die Anlegung eines Reservoirs durch Ausfällung von Protein mit dieser RNS. Von anderer Seite wird auch die Möglichkeit diskutiert (VINCENT 1955, WOODS und TAYLOR 1959 u. a.), daß der Nukleolenbildungsort überhaupt keiner eigenen Syntheseleistung fähig ist, sondern

lediglich als („organizer" im Sinne von McClintock) die Ansammlung von fertigem Ribonukleoprotein katalysiert. Man hat zwei Denkmöglichkeiten: 1. daß die am Genom als „templates" entstandenen RNS-Moleküle nicht oder nicht unversehrt ins Plasma gelangen können, bevor sie am Nukleolenbildungsort mit Trägerprotein verkoppelt sind, oder 2., daß der Nukleolenbildungsort nur die Überschüsse an RNS durch Verkopplung mit dem nukleolären Protein zeitweilig aus dem Verkehr zieht. Das Fehlen des Nukleolenbildungsortes hätte nach der ersten Version das allmähliche Aufhören der gesamten Proteinsynthese zur Folge; nach der zweiten, der Reservoirhypothese in reiner Form, wäre mit Störungen in der Koordination der verschiedenen Syntheseprozesse zu rechnen, als Folge von Unregelmäßigkeiten im Angebot der RNS bzw. des nukleolären Proteins.

Ob die Entstehung des Nukleolus eine eigene Syntheseleistung des Nukleolenbildungsortes voraussetzt und welcher Art diese Syntheseleistung ist, kann nur experimentell entschieden werden. Aus einer sorgfältigen mikrophotometrischen Untersuchung an verschiedenen aberranten Genotypen vom Mais (Polyploide, Polysome mit verschiedenen Anzahlen von B-Chromosomen) hat Mei Lin (1955) als erster geschlossen, daß der Nukleolenbildungsort den RNS-Gehalt der Nukleolen entscheidend beeinflußt: Die absolute Menge der RNS pro Nukleolus erwies sich allein abhängig davon, wie viele Nukleolenbildungsorte an der Herstellung des Nukleolus mitwirkten, gleichgültig wie viele und welche Chromosomen der Kern sonst enthielt. Vincent (1955) fand, daß die RNS der Nukleolen von Seesternoocyten sich von der übrigen RNS der Oocyte in der Basenzusammensetzung unterscheidet (mehr Guanin, weniger Uracil). Pelling (1959) konnte zeigen, daß bei *Chironomus* der Einbau von radioaktivem Uridin in die Nukleolen stets vom Nukleolenbildungsort ausgeht; der Einbau beginnt praktisch gleichzeitig mit dem Einbau an den übrigen RNS-synthetisierenden Chromosomenorten. Diese Befunde sprechen dafür, daß der Nukleolenbildungsort seine RNS selbst herstellt.

In diesem Zusammenhang könnte es auch interessant sein, Situationen oder Bedingungen zu finden, unter denen der Bildungsort nachweislich seine normalen Syntheseleistungen vollbringt, ein Nukleolus aber nicht gebildet wird, etwa weil die Nukleolensubstanz im Augenblick ihrer Entstehung sich gleich im Kernsaft verteilt oder löst. Näherungsweise mag dieser Zustand verwirklicht sein in solchen Zellkernen, die an Stelle eines einheitlichen Nukleolus zahlreiche Bröckchen nukleolären Materials enthalten. In den Versuchen von McClintock (1934) trat eine Klasse von Mikrosporen auf, deren Genom eine große Defizienz aufwies, und deren Kerne trotz Anwesenheit eines Nukleolenbildungsortes nur verstreute Bröckchen von Nukleolensubstanz enthielten. Jain (1957) erhielt das gleiche Ergebnis in prämeiotischen Interphasekernen von *Lolium*, wenn er die Pflanzen 38 Std oder länger einer Temperatur von 35° C ausgesetzt hatte. Beide Autoren deuten ihre Befunde im Sinne einer „Inaktivierung" des Nukleolen-

bildungsortes, obwohl dessen Syntheseaktivität nicht unbedingt beeinträchtigt zu sein braucht. Es ist an sich denkbar, daß die normale Nukleolensubstanz unter extremen Bedingungen die Fähigkeit verliert, zu einem einheitlichen großen Nukleolus zusammenzutreten. Diese Wirkung scheint in Gewebekulturen die Zugabe von Adenosin zu haben (HUGHES 1952, LETTRÉ und SIEBS 1955). Merkwürdig bleibt allerdings die Beobachtung von JAIN, daß sich in den aberranten Pollenmutterzellen nur diejenigen Bivalente normal kontrahieren, denen ein „nucleolar body" anhaftet; vielleicht bestehen die „nucleolar bodies" doch nicht aus normaler Nukleolensubstanz.

Jede Hypothese, die eine eigene synthetische Leistung des „organizer" verneint, muß sich im übrigen mit der Tatsache auseinandersetzen, daß es Nukleolenbildungsorte gibt, die sozusagen „sinnlose" Nukleolen produzieren, wie den am Chromosom 4 von *Chironomus tentans* und *C. pallidivittatus* gebildeten „Sondernukleolus". Dieser Sondernukleolus enthält RNS und Protein, kann aber die normalen Nukleolen physiologisch nicht vertreten. Nimmt man an, daß alle Nukleolenbildungsorte in grundsätzlich gleicher Weise funktionieren, und fordert man, daß diese Funktion sich in der Ansammlung von anderweitig gebildetem Ribonukleoprotein erschöpft, so ist man gezwungen, verschiedenwertigen Nukleolenbildungsorten ein Wahlvermögen zwischen verschiedenartigen Ribonukleoproteinen zuzuschreiben. Auch hier ist die einfachere Alternative die, daß die Nukleolenbildungsorte ihre eigene RNS herstellen, und daß verschiedenwertige Nukleolen durch verschiedene Syntheseleistungen der Bildungsorte zustande kommen.

Zu dem Problem der Nukleolenfunktion können auch die Befunde über die Struktur der Nukleolenbildungsorte, und insbesondere die Fragmentationsversuche, beitragen. Bereits MCCLINTOCK (1934) hatte eine röntgeninduzierte Chromosomenmutation beschrieben, durch welche der „nucleolar organizer" von *Zea mays* in zwei funktionsfähige Teilstücke zerlegt worden war; auch ROMAN (zit. nach MEI LIN 1955) konnte eine derartige Fragmentation erzielen. Im Hinblick auf die relativ zum gesamten Chromosom 6 beträchtliche Länge des von MCCLINTOCK als „organizer" definierten heterochromatischen Segments überrascht die Möglichkeit der Unterteilung nicht. Die Befunde lassen auch die Deutung zu, daß der „organizer" vom Mais in Wahrheit aus zwei eng benachbarten Nukleolenbildungsorten besteht. Ähnlichen formalen Einwänden unterliegen die bisher berichteten Fälle von Fragmentation des Nukleolenbildungsortes bei *Drosophila melanogaster* und anderen *Drosophila*-Species (KAUFMANN 1938, KRIVSHENKO 1959), weil die Lage der „organizer"-Region im Heterochromatin ihre exakte cytologische Definition erschwert (vgl. COOPER 1959). Bei *Chironomus* dagegen ist der Nukleolenbildungsort ein seiner Lage und Struktur nach scharf abgegrenzter und in sich einheitlicher Chromosomenabschnitt von weniger als 1% der Gesamtlänge eines Speicheldrüsenchromosoms. Auch in der

Mitose und Meiose von *Chironomus* sind homologe Chromosomen mit und ohne Nukleolenbildungsort gleich lang. Daß es gelingt, einen derartig kurzen als Einheit fungierenden Abschnitt, der womöglich in einem einzigen Mutationsschritt entstehen oder verschwinden kann (BEERMANN 1955), relativ oft (2 Fälle unter etwa 200 induzierten Chromosomenmutationeñ bei *Chironomus*, 6 verschiedene Brüche bei *Drosophila buscki*, vgl. KRIVSHENKO 1959) in ungleiche, vielleicht beliebige Teilstücke zu zerlegen, die jedes für sich (zumindest bei *Chironomus*) zellphysiologisch noch dasselbe leisten wie der ungeteilte Nukleolenbildungsort, spricht für die Zusammensetzung dieses Ortes aus zahlreichen identischen Teilstücken. Vielleicht hängt damit seine eigenartige Struktur in den Riesenchromosomen zusammen, die (abgesehen von der DNS-Armut) der Struktur heterochromatischer Abschnitte nahekommt. Für die am Nukleolenbildungsort entstehenden RNS-Moleküle würde man folgern, daß sie ebenfalls in viele identische Teilstücke zerfallen können oder von vornherein als relativ niedermolekulare Einheiten entstehen. Dies setzt voraus, daß die tatsächliche „Länge", gemessen in der Anzahl der Nukleotidpaare, bei der DNS der Nukleolenbildungsorte größenordnungsmäßig die gleiche ist wie bei den gewöhnlichen „Genen" (als Funktionseinheiten verstanden). Die cytologischen Verhältnisse in den Riesenchromosomen von *Chironomus* widersprechen dem nicht. Weitere Untersuchungen müssen zeigen, wieweit die Unterteilung von Nukleolenbildungsorten ohne Verlust der Funktion getrieben werden kann.

Zusammenfassend läßt sich über die Funktion von Nukleolenbildungsort und Nukleolus die folgende Meinung vertreten: Die Nukleolenbildungsorte sind wie viele andere Chromosomenorte Stätten der RNS-Synthese. Die an den Nukleolenbildungsorten entstehende RNS unterscheidet sich aber von anderen RNS-Typen dadurch, daß sie ein bestimmtes Protein, das in allen Zellen in großen Mengen gebildet wird, in einer für die Zelle jederzeit verfügbaren Form ausfällt. Dadurch entsteht der Nukleolus. Vielleicht ist das ausgefällte Protein das Trägerprotein der RNP-Partikel im Cytoplasma. Vielleicht hat auch die nukleoläre RNS selber noch wesentliche Funktionen im Proteinstoffwechsel zu erfüllen, etwa als niedermolekulare, lösliche RNS. Wesentlich wird hier die Beantwortung der Frage sein, auf welche Weise das dynamische Gleichgewicht zwischen Aufbau und Abbau des Nukleolus, das ja seine Eigenschaft als Speicher bestimmt, zustande kommt. Die abbauenden, auflösenden Prozesse, die wahrscheinlich an der Peripherie des Nukleolus angreifen, sind noch ganz unbekannt. Befunde von HUGHES (1952) sowie LETTRÉ und SIEBS (1955), wonach Adenosin und andere Purinderivate die Nukleolensubsatnz teilweise auflösen, deuten vielleicht hin auf eine Rückkoppelung (feed back) zwischen RNS-Synthese und Proteinsynthese auf dem Weg über den Nukleolus.

Summary

1. The two sibling species, *Chironomus tentans* and *C. pallidivittatus*, differ genetically with respect to the number and the chromosomal location of their nucleolus organizers. These differences are not due to any structural rearrangement of the chromosomes. With respect to location therefore, the two organizers of *C. tentans* are not homologous to the one organizer of *C. pallidivittatus*.

2. Since hybrids are fertile, recombinant genotypes may be obtained which show any combination from 3 homozygous nucleolar organizers to none at all. All but the last are viable and fertile. This demonstrates the functional equivalence of the three organizers. The absence of any of the three organizers as such is not deleterious.

3. A balanced heterozygous strain has been established which regularly segregates approximately 25% "nucleolusless" progeny. The actual segregation data in a cross of nucleolus heterozygotes often deviate significantly from the 1:2:1 ratio in favor of the nucleolus heterozygotes and homozygotes.

4. Nucleolusless zygotes regularly die as embryos. Development ceases to be normal shortly after the formation of the germ band following blastoderm and may be described as a general failure of gastrulation. No endoderm has been found. In spite of the desorganization of the nucleolusless embryo, mitotic division and differentiation of a few cell types may go on.

5. Nuclei of the nucleolusless embryos show numerous small granules of spherical shape instead of the typical, large nucleoli of irregular outline. The distinction is clear even at the beginning of blastoderm when the nucleoli just begin to be formed in the normal embryo.

6. An accessory nucleolus organizer occurs in a mutant 4th chromosome of *C. tentans* and *C. pallidivittatus*. The accessory nucleolus is different in structure from the normal nucleoli and does not fuse with these. When present in an otherwise nucleolusless embryo, the accessory nucleolus is unable to sustain normal development. Such embryos degenerate in the same manner as completely nucleolusless embryos.

7. By irradiation the normal nucleolar organizers may be broken into fragments of different length which, when recombined with other chromosome breaks, still form a nucleolus each. The capacities of two such partial organizers have been tested by introducing them singly into an otherwise nucleolusless genome. Both were shown to fulfill the functions of a complete organizer, so that one partial organizer alone was sufficient to sustain normal development.

8. The possible functional significance of organizers and nucleoli is considered. It is suggested that the nucleolus organizer synthesizes a special type of RNA. One of the functions of this RNA would be to precipitate and temporarily store a protein of basic importance for all types of „growth", perhaps the carrier protein of the ribosomes (as suggested by BRENNER 1959). The nucleolus may be an essential link in a feed back mechanism involving RNA-synthesis and protein-synthesis.

Literatur

ACTON, A. B.: The gene contents of overlapping inversions. Amer. Naturalist **92**, 57—58 (1958).

BAUER, H.: Der Aufbau der Chromosomen aus den Speicheldrüsen von *Chironomus Thummi* KIEFER. Z. Zellforsch. **23**, 280—313 (1935). — Beiträge zur vergleichenden Morphologie der Speicheldrüsenchromosomen. Zool. Jb., Abt. allg. Zool. u. Physiol. **56**, 239—276 (1936).

BEERMANN, W.: Chromomerenkonstanz und spezifische Modifikationen der Chromosomenstruktur in der Entwicklung und Organdifferenzierung von *Chironomus tentans*. Chromosoma (Berl.) **5**, 139—198 (1952). — Chromosomenpolymorphismus und Bastardierung zweier *Chironomus*-Arten. Zool. Anz. Suppl. **17**, 290—295 (1953). — Cytologische Analyse eines *Camptochironomus*-Artbastards. I. Kreuzungsergebnisse und Evolution des Karyotypus. Chromosoma (Berl.) **7**, 198—259 (1955). — Diskussionsbeitrag in C. H. WADDINGTON, Biological organization. London 1959.

—, and G. F. BAHR: The submicroscopic structure of the Balbiani-ring. Exp. Cell Res. **6**, 195—201 (1954).

BORSTEL, R. C. v., and M. L. REKEMEYER: Division of a nucleus lacking a nucleolus. Nature (Lond.) **181**, 1597—1598 (1958).

BRENNER, S.: In C. H. WADDINGTON, Biological organisation, p. 98. London: Pergamon Press 1959.

CASPERSSON, T.: Cell growth and cell function. New York 1950.

COOPER, K. W.: Cytogenetic analysis of major heterochromatic elements (especially Xh and Y) in *Drosophila melanogaster*, and the theory of "Heterochromatin". Chromosoma (Berl.) **10**, 535—588 (1959).

ELSDALE, T. R., M. FISCHBERG and S. SMITH: A mutation that reduces nucleolar number in *Xenopus laevis*. Exp. Cell Res. **14**, 642—643 (1958).

GAULDEN, M. E., and R. P. PERRY: Influence of the nucleolus on mitosis as revealed by ultraviolet microbeam irradiation. Proc. nat. Acad. Sci. (Wash.) **44**, 553—559 (1958).

HEITZ, E.: Die Ursache der gesetzmäßigen Zahl, Lage, Form und Größe pflanzlicher Nukleolen. Planta (Berl.) **12**, 775—844 (1931a). — Nukleolen und Chromosomen in der Gattung *Vicia*. Planta (Berl.) **15**, 495—505 (1931b).

HUGHES, A.: The mitotic cycle. London 1952.

JAIN, H. K.: Effect of high temperature on meiosis in *Lolium*: Nucleolar inactivation. Heredity **11**, 23—36 (1957).

KAUFMANN, B. P.: Nucleolus organizing regions in salivary gland chromosomes of *Drosophila melanogaster*. Z. Zellforsch. **28**, 1—11 (1938).

Krivshenko, J.: The divisibility of the nucleolus organizer and the structure of the nucleolus in *Drosophila buscki*. Genetics **44**, 520—521 (1959).

Lettré, R., u. W. Siebs: Beobachtungen über die Struktur des Nukleolus in normalen Zellen sowie in Tumorzellen. Z. Krebsforsch. **60**, 564—580 (1955).

McClintock, B.: The relation of a particular chromosomal element to the development of the nucleoli in *Zea mays*. Z. Zellforsch. **21**, 294—328 (1934).

Mei Lin: Chromosomal control of nucleolar composition in maize. Chromosoma (Berl.) **7**, 340—370 (1955).

Pelling, C.: Chromosomal synthesis of ribonucleic acid as shown by the incorporation of uridine labeled with tritium. Nature (London) **184**, 655—656 (1959).

Vincent, W. S.: Structure and chemistry of nucleoli. Int. Rev. Cytol. **4**, 269—298 (1955).

Woods, P.S., and J.H. Taylor: Studies of ribonucleic acid metabolism with tritium-labeled cytidine. Lab. Invest. **8**, 309—318 (1959).

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